Exhibit 69

Retrograde Menstruation in Healthy Women and in Patients With Endometriosis

JOUKO HALME, MD, PhD, MARY G. HAMMOND, MD,

JAROSLAV F. HULKA, MD, SHAILAJA G. RAJ, MD,

AND LUTHER M. TALBERT, MD

Blood was found in the peritoneal fluid in 90% of women with patent tubes at laparoscopy during perimenstrual time. If the fallopian tubes were occluded, then only 15% of patients had evidence of blood in the pelvis. Also, 90% of patients with endometriosis and eight of nine women on oral contraceptives had bloody fluid during the menstrual period. The present observations indicate that retrograde menstruation through the fallopian tubes into the peritoneal cavity is a very common physiologic event in all menstruating women with patent tubes. (Obstet Gynecol 64:151, 1984)

In 1927 Sampson proposed that endometriosis was due to implantation of endometrial cells during retrograde menstruation.[1] During his lifetime, most of the opponents of this theory dismissed it mainly on the basis that retrograde menstruation, although occasionally noted to occur, was a relatively rare phenomenon.[2,3] Therefore, it would not explain the development of a common clinical entity such as endometriosis. Since that time, the frequency of retrograde menstruation has been debated.

No systematic studies documenting the incidence of retrograde menstruation have been published in spite of the fact that millions of women have undergone laparotomy or laparoscopy, making possible direct observations of pelvic structures. Recently, however, Blumenkrantz et al[4] reported that nine of 11 menstruating women undergoing peritoneal dialysis had blood present regularly in the dialysate during the time of their period and in this way documented retrograde menstruation. They also suggested that this event was a rather common phenomenon, and not limited to women with renal failure. In addition, a study from the authors' institution reported that of 80 peritoneal fluid samples, all four obtained during menses were bloody.[5]

Based on laparoscopy of 323 women, the current study presents further evidence suggesting that retrograde menstruation occurs in most menstruating women who have open fallopian tubes.

Material and Methods

Between July 1980 and September 1983, 331 pelvic fluid samples were obtained from patients undergoing laparoscopy at The North Carolina Memorial Hospital. Of

181 patients with patent tubes and normal pelves, 78 underwent laparoscopy for bilateral tubal ligation, and 103 were undergoing diagnostic laparoscopy for evaluation of infertility or chronic pelvic pain. Of 40 patients with occluded fallopian tubes, 16 had distal blockage, two had proximal blockage, and 22 had proximal occlusion as a result of previous tubal ligation. Eighty-one patients were noted to have mild to moderate endometriosis.

Peritoneal fluid was aspirated with an 18-gauge Silastic catheter through the operative channel of the laparoscope and collected into a heparin-containing test tube. The color of the fluid, when in the tube, was recorded either as straw, pink, or bloody. Upon reviewing the records of these patients, the date of the last normal menses in 302 patients, and observations on the fluid samples were available. In addition, 21 women who were on oral contraceptives were identified.

Because only visual documentation of the color of the fluid was available for all samples, an experiment was set up to test the accuracy of this technique in assessment of the presence of blood. A series of 30 tubes containing ten different concentrations of red blood cells (ranging from hematocrit of 0 to 10) in peritoneal fluid was constructed. The tubes were shown in a random order to each of the nine persons involved in classifying these fluids into one of the three color categories. The color was judged as straw when hematocrit was less than 0.5 ± 0.2% (SD) and bloody when hematocrit was higher than 3.2 ± 2.0%. Between these values, the color was judged to be pink. The level of agreement between different individuals and by each individual between two testing occasions (the coefficient κ) was determined according to Cohen[6]

and Fleiss.[7] Values of κ ranged from 0.39 to 1.0 between pairs of individuals and from 0.52 to 1.0 between the two testings.

Observations by Blumenkrantz et al[4] suggested that the presence of blood in the peritoneal dialysates usually preceded the beginning of menstrual flow by one to two days. Therefore, the patients in this series were divided in two groups: 1) perimenstrual, if they underwent laparoscopy on days one to six or 27 to 30 of their cycle, and 2) nonmenstrual, if laparoscopy was performed between days 7 to 26 of the cycle.

Statistical analysis of the data was performed by using the χ^2 statistic for 2 × 2 contingency tables constructed for pairs of variables and normal approximation for the binomial distribution.[8]

Results

Figure 1 presents the noted color of each of the 302 fluid samples in perimenstrual and nonmenstrual phases of the cycle. It is obvious from the graph that there is an increased amount of blood in the pelvic cavity around the time of menses and also immediately after ovulation with clearance of that blood over the next five to six days.

As indicated in Table 1, a total of 237 fluid samples were obtained in the nonmenstrual phase. Overall one-third of these fluids were straw, and the other two-thirds contained an appreciable amount of red blood cells (either pink or bloody). In normal women with open fallopian tubes, 61.1% of fluids were either pink or bloody as compared with 60% in women with occluded tubes, suggesting that tubal patency is not an important factor for the presence of blood in the peritoneal cavity during the nonmenstrual phase of the

Table 1. Appearance of Peritoneal Fluid Samples Obtained on Nonmenstrual Days 7 to 26

	No endometriosis		Endometriosis
	Open tubes	Closed tubes	
Straw	54	11	14
Pink or bloody	85	16	57
Total	139	27	71

Table 2. Appearance of Peritoneal Fluid Samples Obtained on Perimenstrual Days 1 to 6 and 27 to 30

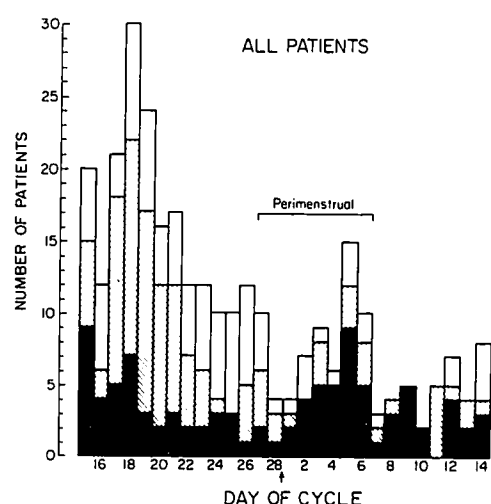|	No endometriosis		Endometriosis
	Open tubes	Closed tubes	
Straw	4	11	1
Pink or bloody	38	2	9
Total	42	13	10

Figure 1. Appearance of all 302 peritoneal fluid samples obtained during laparoscopy. Solid bars = bloody; shaded bars = pink; open bars = straw.

normal cycle. In the 12 women on oral contraceptives who underwent laparoscopy during this phase of the cycle, six had pink fluid in the cul-de-sac. In patients with endometriosis, blood was detected significantly more often ($P \leq .005$) than in other women with patent tubes in the nonmenstrual phase.

Table 2 presents corresponding data for fluid samples obtained during the perimenstrual phase of the cycle. Of 52 samples from women with patent fallopian tubes, 47 (90.4%) had an appreciable amount of red blood cells; 70% of these were grossly bloody. This is significantly different ($P \leq .001$) than the corresponding percentage in nonmenstrual samples. In the nine women on oral contraceptives who underwent laparoscopy in the perimenstrual phase, eight had bloody fluid. Only two of 13 (15.4%) patients with occluded tubes had red blood cells (one pink and one bloody sample) in the peritoneal fluid. This frequency is significantly lower ($P \leq .005$) than in women with open tubes. These figures clearly indicate that during the perimenstrual phase, the peritoneal fluid in almost all women, including those taking oral contraceptives, contains blood and that the fallopian tubes play an important role as conduits for menstrual blood.

Discussion

The important clinical observations by Blumenkrantz et al[4] in women undergoing peritoneal dialysis indicated that bleeding into the dialysate usually was detectable one to two days before the menstrual period and during the menses. The recognition of this phenomenon prompted the authors to include the patients undergoing laparoscopy on these premenstrual days in the perimenstrual group rather than in the nonmenstrual group. The results of this study clearly indicate that during this perimenstrual time of the cycle, over 90% of normal and infertile women have blood in their peritoneal fluid. If the tubes are occluded, there is no correlation between the perimenstrual phase and the presence of blood in the pelvis. This indicates that the fallopian tubes are the major conduit for blood entering the peritoneal compartment at the time of menses.

The use of oral contraceptives has been advocated as a possible means of protection from endometriosis,[5,9] but it may be inferred from the present data that if used noncontinuously, allowing menstruation to occur, retrograde menstruation will also occur, as these women consistently had blood in the pelvic fluid at this time. To prevent this, an uninterrupted mode of administration may be necessary.

Many studies have demonstrated that various volumes of peritoneal fluid are found in the female pelvis during laparoscopy.[5,10,11] This fluid in the pelvis often seems to contain blood.[12,13] In 69% of all patients in this series, an appreciable amount of blood was detected. Sources of this blood include the abdominal wall stab wound(s) and severed vessels in omentum or adhesions in the pelvis. This contamination with fresh blood is always variably present in addition to blood derived from natural, physiologic phenomena like ovulation, and eventually, retrograde menstruation. It is not possible to accurately assess the impact of this contamination, but it may be safe to assume that this iatrogenic hemorrhage occurs at random and is not dependent on any particular time of the cycle. Furthermore, observation (not shown) that even grossly bloody peritoneal fluid samples obtained during menses did not contain appreciable numbers of granulocytes suggests that the blood did not result from an immediate hemorrhage to the pelvic compartment.

Sampson[1] originally suggested that retrograde menstruation provides a mechanism by which endometrial cells can implant on peritoneal surfaces in women with endometriosis. Because the great majority of the authors' patients either with or without endometriosis showed evidence of retrograde menstruation, it cannot explain why only some women have developed the disease. Other factors, either hormonal or immunologic, will apparently determine whether or not ectopic implantation can take place. Koninckx et al[14,15] demonstrated a high incidence of luteinized unruptured follicle syndrome in women with endometriosis, and also a low, late luteal phase progesterone/estrogen ratio of peritoneal fluid in this syndrome. They hypothesized that this local hormonal imbalance may be critical in allowing endometrial cells, if present in the peritoneal compartment, to implant on the peritoneum. The results of the present study provide direct evidence that cells originating from the uterine cavity indeed are present in the pelvis in the late luteal phase preceding menses, and this theory may hold if peritoneal fluid hormone levels are abnormal. However, a recent study[16] found no difference in progesterone and estrogen levels during this time in the fluid of women with or without endometriosis. However, several sources[17,18] suggest that abnormal immunologic defense mechanisms may be operative in women with endometriosis, and this can explain the occurrence of ectopic implantation of endometrium. More detailed comparative information on both hormonal and immunologic function in a sizeable population of both normal women and patients with endometriosis is clearly warranted.

Studies on peritoneal macrophages[5,19] have demonstrated that samples taken at menstruation usually have the highest concentrations of these cells, the majority of which are recent arrivals. It was suggested that this influx of phagocytic macrophages to the pelvis

is a response to retrograde menstruation. The present results clearly support this idea, and it remains to be seen whether or not the nucleated cellular components of menstrual detritus are also regularly transported through the fallopian tubes. Studies are in progress in The North Carolina Memorial Hospital to detect the presence of either epithelial or stromal cells of endometrial origin in peritoneal fluid.

References

1. Sampson JA: Peritoneal endometriosis due to the menstrual dissemination of endometrial tissue into the peritoneal cavity. Am J Obstet Gynecol 14:422, 1927
2. Novak E: Pelvic endometriosis and its treatment. Am J Surg 33:422, 1936
3. Watkins RE: Uterine replacement, retrograde menstruation and endometriosis. West J Surg 46:480, 1938
4. Blumenkrantz MJ, Gallagher RN, Bashore RA, et al: Retrograde menstruation in women undergoing chronic peritoneal dialysis. Obstet Gynecol 57:667, 1981
5. Halme J, Becker S, Hammond MG, et al: Pelvic macrophages in normal and infertile women: The role of patent tubes. Am J Obstet Gynecol 142:890, 1982
6. Cohen J: A coefficient of agreement for nominal scales. Educat Psychol Measure 20:37, 1960
7. Fleiss JL: Measuring nominal scale agreement among many raters. Psychol Bull 76:379, 1971
8. Remington RD, Schork MA: Statistics with Applications to the Biological and Health Sciences. Englewood Cliffs, NJ, Prentice-Hall, 1970, pp 229–246
9. Kistner RW: Endometriosis and infertility. Clin Obstet Gynecol 22:101, 1979
10. Maathuis JB, Van Look PFA, Michie EA: Changes in volume, total protein and ovarian steroid concentration of peritoneal fluid throughout the human menstrual cycle. J Endocrinol 76:123, 1978
11. Koninckx PR, Renaer M, Brosens IA: Origin of peritoneal fluid in women: An ovarian exudation product. Br J Obstet Gynaecol 87:177, 1980
12. Polishuk WA, Sharf M: Culdoscopic findings in primary dysmenorrhea. Obstet Gynecol 26:746, 1965
13. Reti LL, Bryne GA, Davoren RAN: The acute clinical features of retrograde menstruation. Aust N Z J Obstet Gynaecol 23:51, 1983
14. Koninckx PR, DeMoor P, Brosens IA: Diagnosis of the luteinized unruptured follicle syndrome by steroid hormone assays of peritoneal fluid. Br J Obstet Gynaecol 87:929, 1980
15. Koninckx PR, Ide P, Vandenbroucke W, et al: New aspects of the pathophysiology of endometriosis and associated infertility. J Reprod Med 24:257, 1980
16. Crain JL, Luciano AA: Peritoneal fluid evaluation in infertility. Obstet Gynecol 161:159, 1983
17. Dmowski WP, Steele RW, Baker GF: Deficient cellular immunity in endometriosis. Am J Obstet Gynecol 141:377, 1981
18. Halme J, Becker S, Wing RD: Accentuated cyclic activation of peritoneal macrophages in patients with endometriosis. Am J Obstet Gynecol 148:85, 1984
19. Halme J, Becker S, Hammond M, et al: Increased activation of pelvic macrophages in infertile women with mild endometriosis. Am J Obstet Gynecol 145:333, 1983

Address reprint requests to:
Jouko Halme, MD, PhD
Department of Obstetrics and Gynecology
University of North Carolina School of Medicine
Old Clinic Building 226H
Chapel Hill, NC 27514

Exhibit 70

Migration of a Particulate Radioactive Tracer from the Vagina to the Peritoneal Cavity and Ovaries

P. F. VENTER,   M. ITURRALDE

SUMMARY

In this report we describe a radionuclide procedure designed to evaluate the migration of a particulate radioactive tracer from the vagina to the peritoneal cavity and ovaries, as well as the determination of the patency of the pathways between these two extremes of the female reproductive system.

^{99m}Tc-labelled human albumin microspheres (^{99m}Tc-HAM) were deposited in the posterior fornices of 24 patients a day before they were to undergo different gynaecological operations. During this period sequential images were obtained and after the operation radioactivity levels in the removed organs and tissues were counted with a scintillation detector.

In 14 out of 21 cases, the ovaries and fallopian tubes were counted separately from the uterus. Nine were positive (radioactivity levels were sufficiently high in the tubes and ovaries) and 5 were negative (no substantial radioactivity levels could be detected in either the tubes or the ovaries). The 5 negative results all occurred in patients with proved tubal damage as a result of previous infection.

All the results were either true positive or true negative, providing evidence of migration, or obstruction, of ^{99m}Tc-HAM from the vagina through the uterus and tubes to the peritoneal cavity and ovaries.

S. Afr. med. J., **55**, 917 (1979).

In the female, the peritoneal cavity is linked with the outside via the fallopian tubes, the uterus and the vagina, and there is evidence of migration of different substances in either direction. For example, malignant cells from ovarian carcinoma can be demonstrated in the posterior fornix of the vagina.[1] After menstruation the gonococcus can penetrate the cervix and gain access through the uterus and tubes to the peritoneal cavity and ovaries.[2] For pregnancy to occur, spermatozoa have to move up the uterus and the ova down the tube. Retrograde menstruation is also a well-known phenomenon. After insufflation, air and gases pass easily from the vagina into the peritoneal cavity up to the diaphragm. Radio-opaque contrast media are introduced with great ease through the uterus and

tubes into the peritoneal cavity, and tubal patency is easily demonstrated during peritoneoscopy by injection of a dye through the cervix and into the tubes.[3]

Does this also hold for inert chemical substances? Will a chemical substance deposited in the vagina later appear in the peritoneal cavity? Such migration could well explain the aetiological role of chemical substances in certain gynaecological diseases. It has already been suggested that talcum powder is one of these potentially dangerous inert chemical products. Electron micrographic slides of removed human ovaries have shown asbestos particles resting on them, and there is evidence that these particles originated from talc used to dust condoms.[4]

To demonstrate the upward migration of chemical substances we made use of radionuclide imaging and counting techniques.

MATERIAL AND METHODS

The subjects of this study were 24 adult women, both Blacks and Whites, from the Academic Hospitals of the University of the Orange Free State in Bloemfontein. All had been admitted to hospital for elective gynaecological surgical operations (Table I). The radionuclide procedure was explained and the necessary consent obtained

TABLE I. SURGICAL INDICATION AND OPERATIVE PROCEDURE

Number of patients	Surgical indication	Operative procedure
4	Sterilization	Fimbriectomy
7	Ca. breast stage III	Bilateral salpingo-oöphorectomy
1	Ca. breast stage III	Hysterectomy and bilateral salpingo-oöphorectomy
2	Postmenopausal bleeding	Dilatation and curettage
2	Postmenopausal bleeding	Hysterectomy and bilateral salpingo-oöphorectomy
3	Menorrhagia	Dilatation and curettage
4	Menorrhagia	Hysterectomy and bilateral salpingo-oöphorectomy
1	Pelvic infection	Hysterectomy and bilateral salpingo-oöphorectomy

Procedure

The patient was placed in the supine position with the buttocks slightly elevated. The cervix and posterior fornix were exposed with a Cusco vaginal speculum and between 10 and 15 mCi of ^{99m}Tc-labelled human albumin microspheres (HAM) in a volume of less than 3 ml was

Department of Obstetrics and Gynaecology, University of the Orange Free State and Academic Hospitals, Bloemfontein
P. F. VENTER, M.MED. (O. & G.), F.R.C.O.G., *Professor*

Department of Nuclear Medicine, University of the Orange Free State and Academic Hospitals, Bloemfontein
M. ITURRALDE, M.D., D.M., *Professor and Head*

deposited in the posterior fornix. The patient was kept in this position for about 2 hours. The vulva was covered with a sanitary towel, and the legs were pressed together to prevent the radionuclide solution streaming from the vagina and thus lowering count levels.

In a few cases images were obtained, 4 and 24 hours after deposition of the radioactive tracer, with a Nuclear Chicago Pho/Gamma III scintillation camera (Figs 1 and 2). In most cases a count was performed on removed surgical specimens as a whole or separately on the uterus

and adnexae, for 1 000 seconds in a 12,7-cm well scintillation detector. In one case a piece of the anterior peritoneum, fluid from the pouch of Douglas and blood were also included in the count, to determine the possibility of reabsorption into the bloodstream from the vaginal mucosa.

Radiation exposure to the patients was low owing to the short half-life of ^{99m}Tc (6 hours), and in most cases it was almost negligible since the target organs had been surgically removed.
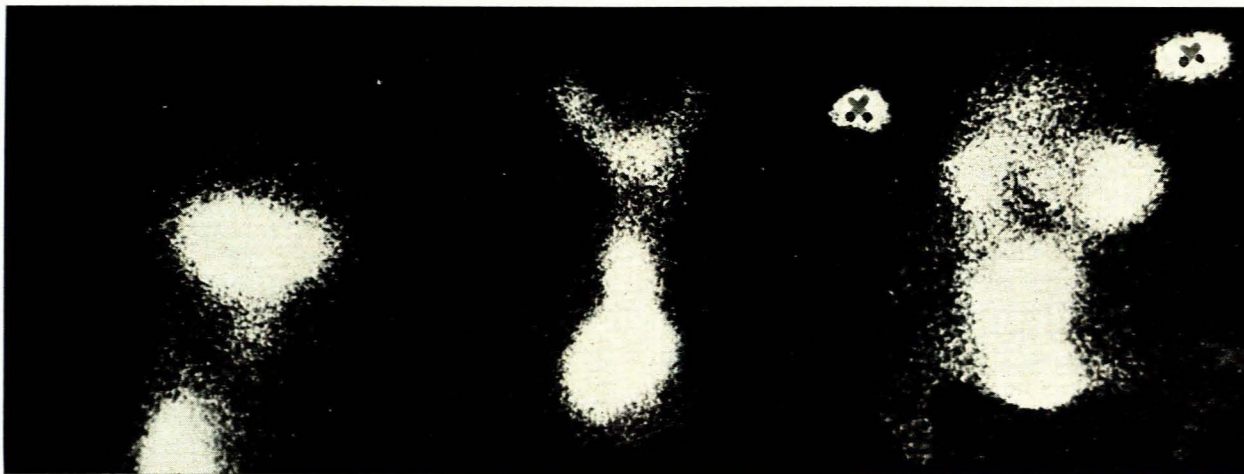

Fig. 1. Scintiphotographs showing positive ^{99m}Tc-HAM migration: A — from the vagina to the uterus (4 hours after deposition); B — in both tubes (6 hours after deposition); C — reaching the peritoneal cavity and ovaries 24 hours after deposition (markers in the anterior superior iliac spines).
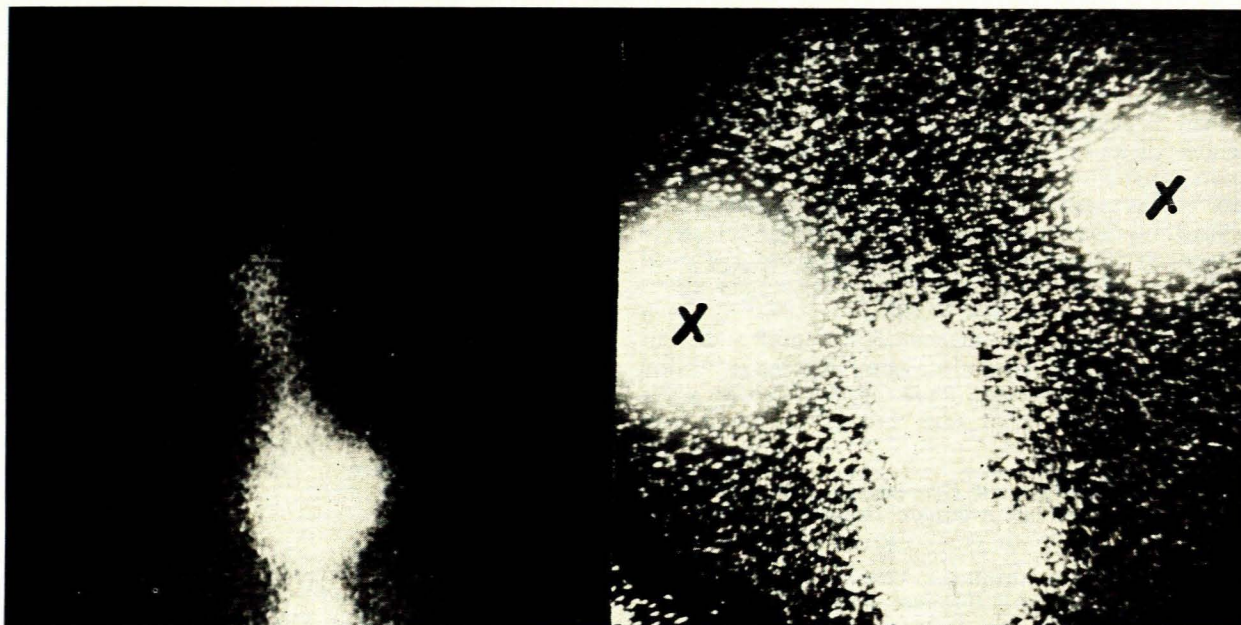

Fig. 2. Scintiphotographs showing negative ^{99m}Tc-HAM migration: A — in the left tube (4 hours after deposition); the right tube is patent; B — in both tubes; 24 hours after deposition radioactivity remains in the uterus (markers in the anterior superior iliac spines).

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RESULTS

A total of 24 patients were examined. Because radio-nuclide material streamed away from the vagina in 3 patients, these cases were considered technically defective and were not included in the final analysis.

Of the remaining 21 cases 16 were positive, that is sufficiently high radioactivity levels were obtained as evidence of migration of the radioactive tracer to the uterus or the tubes and ovaries. The results were negative in 5 cases; in 2 of them the radioactive microspheres did not pass from the vagina to the uterus and in the other 3 there was no migration to the adnexae or fimbria. In the latter, it was impossible to determine radioactivity levels in the uterus because the latter was not removed.

TABLE II. SUMMARY OF RESULTS

Patient	Tissue examined	Radioactivity present (+) or absent (−)
1	Organ imaging fimbria	Uterus, adnexa, fimbria +
2	Organ imaging	Uterus and adnexa +
3	Organ imaging fimbria	Uterus, adnexa, fimbria +
4	Organ imaging adnexa	Uterus +, adnexa +
5	Uterus and adnexa	Uterus +, adnexa −
6	Endometrium	Endometrium −
7	Organ imaging endometrium	Uterus and endometrium +
8	Organ imaging endometrium	Uterus and endometrium −
9	Endometrium	Endometrium +
10	Uterus and adnexa	Uterus and adnexa +
11	Adnexa	Adnexa +
12	Uterus and adnexa	Uterus and adnexa +
13	Uterus and adnexa	Uterus, adnexa +
14	Endometrium	Endometrium +
15	Uterus and adnexa	Uterus +, adnexa −
16	Adnexa	Adnexa +
17	Adnexa	Adnexa +
18	Fimbria	Fimbria −
19	Uterus and adnexa	Uterus and adnexa +
20	Adnexa	Adnexa −
21	Adnexa	Adnexa −

In 14 out of 21 cases it was possible to measure radio-activity levels in the adnexa separately from the uterus. Nine of these showed marked radioactivity in the tubes and ovaries, while in 5 the radioactivity levels were not much higher than the background. In all 5 of these patients, severe tubal occlusion due to previous infection was confirmed by study of the removed specimens (Table II).

In 1 case, radioactivity levels in blood were not much higher than in the background, which indicated that radio-active tracer had not reached the adnexa through the blood supply owing to local reabsorption in the vaginal mucosa.

DISCUSSION

Evidence is available for migration of different substances in either direction within the female reproductive system between the peritoneal cavity and ovaries via the tubes, uterus and vagina, and the outside. Various living organisms actively follow this pathway in both directions. Gases, fluids, dyes and contrast media can easily be introduced from the vagina into the peritoneal cavity. If transit can take place so easily, it is probably the same for many chemical substances used for hygienic, cosmetic or medicinal purposes, many of which may have potential carcinogenic or irritating properties.

To prove this would be of great practical value, because migration of certain chemical substances could play an important aetiological role in gynaecological diseases and especially in carcinoma of the ovary.

We found the use of a particulate radioactive agent such as ^{99m}Tc-HAM with a size range of 30 - 50 μm to be a suitable and safe means of imaging and evaluating tubal patency and demonstrating the possibility of transit of particles from the vagina to the peritoneal cavity and ovaries.

Results obtained by this technique correlated with findings in the surgically removed specimens, thus demonstrating the accuracy of this radionuclide procedure.

REFERENCES

1. Graham, R. and Graham, R. C. (1967): Brit. J. Obstet. Gynaec., 74, 371.
2. Schwarz, R. H. in Monet, G. R. G., ed. (1974): Diseases in Obstetrics and Gynaecology, pp. 381 - 395. London: Harper & Row.
3. Jordan, J. A. (1974): Clinics Obstet. Gynaec., 1, 395.
4. News and Comment (1978): S. Afr. med. J., 54, 14.

Exhibit 71

THE UTERINE PERISTALTIC PUMP

Normal and Impeded Sperm Transport within the Female Genital Tract

G. Kunz,[1] D. Beil,[2] H. Deiniger,[2] A. Einspanier,[4] G. Mall,[3] and
G. Leyendecker[1]

[1]Department of Obstetrics and Gynecology
[2]Department of Radiology I
[3]Department of Pathology
 Klinikum Darmstadt
 Academic Teaching Hospital to the University of Frankfurt
 Grafenstr. 9, 64283 Darmstadt, Germany
[4]Deutsches Primatenzentrum GmbH
 Kellnerweg 4, 37077 Göttingen, Germany

1. SUMMARY

Rapid as well as sustained sperm transport from the cervical canal to the isthmical part of the fallopian tube is provided by cervico-fundal uterine peristaltic contractions that can be visualized by vaginal sonography. The peristaltic contractions increase in frequency and presumably also in intensity as the proliferative phase progresses. As shown by placement of labeled albumin macrospheres of sperm size at the external cervical os and serial hysterosalpingoscintigraphy (HSSG) sperm reach, following their vaginal deposition, the uterine cavity within minutes. In the early follicular phase a large proportion of the macrospheres remains at the site of application, while a smaller proportion enters the uterine cavity with even a smaller one reaching the isthmical part of the tubes. In the mid-follicular phase of the cycle with increased frequency and intensity of the uterine contractions the proportion of macrospheres entering the uterine cavity as well as the tubes has significantly increased. In the late follicular phase with maximum frequency and intensity of uterine peristalsis the proportion of macrospheres entering the tube increases further at the expense of those at the site of application as well as within the uterine cavity. The transport of the macrospheres into the tube is preferentially directed into the tube ipsilateral to the dominant follicle, which becomes apparent in the mid-follicular phase as soon as a dominant follicle can be identified by ultrasound. Since the macrosphere are inert particles the directed sperm transport into the tube ipsilateral to the dominant follicle is not

functionally related to a mechanism such as chemotaxis but is rather provided by uterine contraction of which the direction may be controlled by a specific myometrial architecture in combination with an asymmetric distribution of myometrial oestradiol receptors.

Women with infertility and mostly mild endometriosis display on VSUP a uterine hyperperistalsis with nearly double the frequency of contractions during the early and mid- as well as midluteal phase in comparison to the fertile and healthy controls. During midcycle these women display a considerable uterine dysperistalsis in that the normally long and regular cervico-fundal contractions during this phase of the cycle have become more or less undirected and convulsive in character. Hyperperistalsis results in the transport of inert particles from the cervix into the tubes within minutes already during the early follicular phase, and may therefore constitute the mechanical cause for the development of endometriosis in that it transports detached endometrial cells and tissue fragments via the tubes into the peritoneal cavity. Moreover, dysperistalsis may contribute to the infertility in these patients since it results in a break down of sperm transport within the female genital tract.

2. INTRODUCTION

There is no doubt that the ultimate fate of the successful male germ cell is to impregnate the female oocyte, where its genetic material will fuse with that of the oocyte to result in fertilization and embryo formation. This particular sperm is usually deposited five to zero days prior to ovulation (Wilcox et al., 1995) in the posterior vaginal fornix in close vicinity to the external os of the cervical canal from where it reaches its final site of destination, the place of sperm-oocyte encounter within the tube ipsilateral to the dominant follicle.

Usually, the uterus is considered to be specialized, first, for the reception of the blastocyst by the endometrium and the continuous nourishment of the developing fetus and, second, for the eventual expulsion of the fetus (Romanini, 1994). Considering the fact that the sperm has to cover a long distance within the female genital tract from the external os of the cervix to the site of fertilization within the tube it is surprising that the facilitation and the guidance of this journey has only recently been considered a genuine and active function of the uterus (Kunz et al., 1996a). Previously, the ascension of the sperm within the female genital tract to the site of fertilization was regarded more or less a functional capacity of the sperm itself with the uterus serving merely as a passive canal, although a functional importance had been ascribed to uterine contractions (Moghissi, 1977; Harper, 1994). With respect to sperm ascension, attention was mostly directed towards the cervical canal with its glands and cyclically changing secretion. These are assumed to provide optimal conditions for the penetration of the sperm into the female genital tract around ovulation and serve, with its crypts, as a sperm reservoir, from where constant release for sustained sperm transport could occur. In this communication, the mechanism of uterine peristalsis will be discussed, and its role in sperm transport within the female genital tract will be outlined. It will be demonstrated that this function of the uterus is of fundamental importance in the process of reproduction and that disturbances of the uterine mechanism of sperm transport may result in infertility.

3. UTERINE PERISTALSIS

Rapid sperm transport from the vagina to the Fallopian tubes within minutes has been described in many species including man (Hartman, 1962; Mortimer, 1983; Hunter,

1987; Drobnis and Overstreet, 1992; Harper, 1994). Since the velocity of sperm move-
ment does not itself account for covering such a long distance through the female genital
tract within a few minutes, rapid sperm transport is considered a passive phenomenon and
has been ascribed to uterine contractions (Moghissi, 1977; Harper, 1994; Kunz et al.,
1996a; Leyendecker et al., 1996).

3.1. Vaginal Sonography of Uterine Peristalsis (VSUP)

Contractile activity of the non-pregnant uterus has been known for many decades
(Hendricks, 1966; Cibils, 1967; Martinez-Gaudio et al., 1973). High resolution sonogra-
phy has made it possible to demonstrate these contractions without invasive techniques.
These contractions involve mostly the subendometrial layer of the myometrium and may
be detected only by endometrial movements (Birnholz, 1984). Following their first de-
scription they have been further characterized (Oike et al., 1988; De Vries et al., 1990;
Lyons et al., 1991; Fukuda and Fukuda, 1994). The contractions increase in frequency and
in intensity as the follicular phase progresses with an inverse pattern during the luteal
phase. The peristaltic waves of the endometrium and the subendometrial layer of the
myometrium are directed from the cervical canal to the fundal part of the uterus, while
only during menstruation do they exhibit a fundo-cervical direction (Lyons et al., 1991).
In our own study (Kunz et al., 1996a; Leyendecker et al.,1996), with measurements
of the uterine peristalsis during the menstrual period, the early, mid- and late follicular as
well as mid-luteal phases of the cycle, respectively (Fig. 1) it was demonstrated that there
was a steady increase in peristaltic activity ranging from roughly 1.2 contraction per m-
inute during the menstrual period and early follicular phase to 2.8 contractions per minute
in the late follicular phase. During the mid-follicular and mid-luteal phases, respectively,
the frequency averaged 1.5 contractions per min. Over the same time period, the propor-
tion of fundo-cervical contraction waves decreased significantly from 43% during the
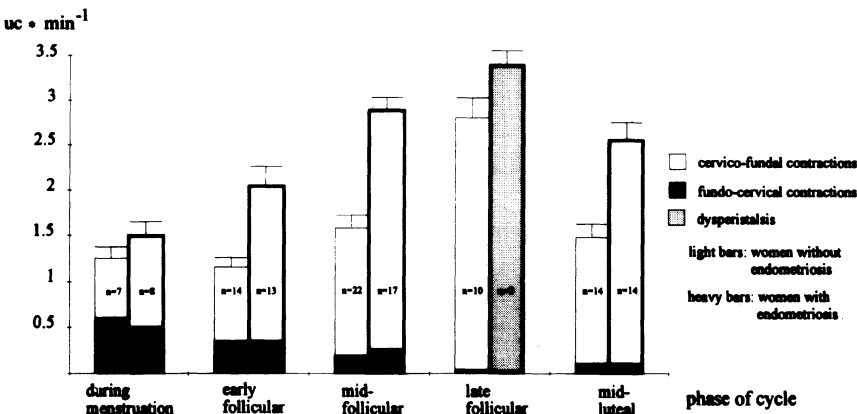menstrual period to less than 1% in the periovulatory phase. Thus, almost all peristaltic

Figure 1. A graphical demonstration of the frequency of the subendometrial uterine peristaltic waves during men-
struation, the early, mid- and late follicular and mid-luteal phases of the cycle, respectively, as determined by
vaginal ultrasonography (contractions/min ± SEM). The graph shows also the relative distribution of fundo-cervi-
cal contractions versus cervico-fundal contractions during these different phases of the cycle (from Leyendecker
et al., 1996).

waves of the uterus during the late follicular phase had a cervico-fundal direction. They also appeared to be more intense than during the other parts of the follicular phase, which might, however, be related to the thickness of the endometrium rendering the movements more pronounced. In comparison to the early follicular phase, however, a thicker proportion of the myometrium appeared also to be involved in the contractions. Because the frequency, intensity and direction of the uterine peristalsis depend upon the phase of the cycle, an endocrine control of this phenomenon by the ovary may be assumed. In this regard oxytocin and prostaglandins may function as mediators (Eliasson and Posse, 1960; Hein et al., 1973; Karim et al., 1973; Fuchs et al., 1985; Lefebvre et al., 1994a, Lefebvre et al., 1994b). This view is supported by the finding that administration of oestradiol valeriate to hypogonadal women yielding a pattern of serum oestradiol values similar to that of the normal cycle could completely mimic the cyclic changes of uterine peristalsis and that the frequency of the uterine peristaltic contractions could be significantly increased during the follicular phase of the cycle by the administration of an i.v. bolus of oxytocin. The increase in the frequency of the peristaltic contractions could be totally attributed to the peristaltic waves with cervico-fundal direction, which may be related to the high density of oxytocin receptors in the cervical tissue (unpublished).

Peristaltic contractions with the same frequency as described above were also observed with transvesical scanning (Birnholz, 1984). Thus, it is very unlikely that the uterine peristalsis was induced by the vaginal ultrasound examination. In contrast, it can be assumed that uterine peristalsis during the menstrual cycle is a continuous phenomenon with varying frequency, intensity and direction of the contraction waves depending on the phase of the cycle and does not require a specific stimulus for initiation. These studies, however, do not exclude a coital enhancement of uterine contractions.

3.2. Hysterosalpingoscintigraphy (HSSG)

It is reasonable to assume that the uterine peristaltic activity provides the forces that are required for the transport of spermatozoa from the external os of the cervix into the tubes within minutes. Using hysterosalpingoscintigraphy (Itturalde and Venter, 1981; Becker et al., 1988), rapid sperm transport was studied by placing technitium-labelled albumin macrospheres of sperm size at the external os of the uterine cervix and following their path through the female genital tract (Kunz et al., 1996a; Leyendecker et al., 1996).The albumin macrospheres used in our study resemble spermatozoa in their size. Thus, the demonstration of their ascension through the genital was considered to represent passive sperm transport.

According to these data (Fig. 2–4) the following concept of the dynamics of rapid sperm ascension within the female genital tract was proposed. Rapid sperm ascension occurs immediately following deposition of the ejaculate at the external os of the cervix. As early as one minute thereafter spermatozoa have reached the intramural and isthmical part of the tube. Quantitatively, however, the extent of ascension increases with the progression of the follicular phase. While only a few spermatozoa enter the uterine cavity and even fewer the tubes during the early follicular phase, the proportion of spermatozoa that enters the uterine cavity increases dramatically during the mid-follicular phase with still a limited entry into the tube. During the late follicular phase there is a considerable ascension of spermatozoa into the tubes.

Furthermore the HSSG revealed the preferential direction of rapid sperm transport into the tube ipsilateral to the dominant follicle. This corresponds with recent findings during surgery that the number of sperm around ovulation was higher in the tube ipsilat-
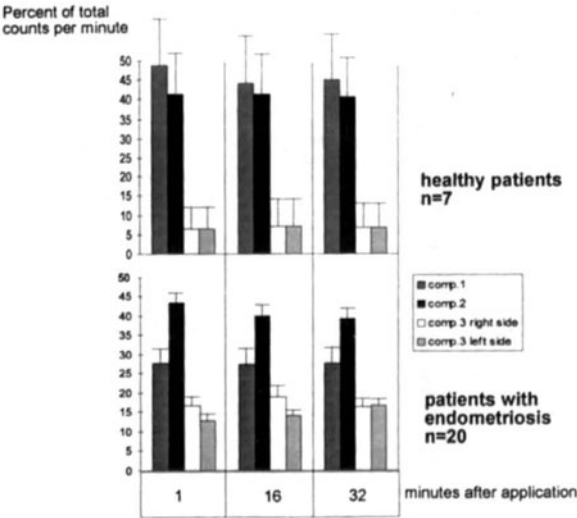
The Uterine Peristaltic Pump

Figure 2. The distribution of the percentage of total counts, representing the labeled albumin macrospheres, within the female genital tract (compartment 1, 2 and 3 being the upper vagina, the uterine cavity and the isthmical part of the tubes respectively) following 1, 16 and 32 minutes after vaginal application during the early follicular phase. With respect to compartment 3, the right and left tubes were differentiated. The amount of radioactivity transported into the tubes was significantly higher in patients with endometriosis in comparison with healthy controls (P< 0.01) (from Leyendecker et al., 1996).
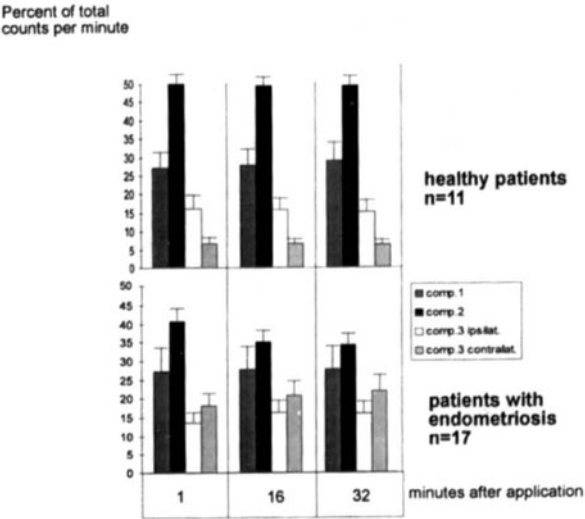

Figure 3. The respective distribution pattern of radioactivity, as in Fig. 2, obtained during the mid-follicular phase of the cycle. While in patients without endometriosis the labeled macrospheres preferentially entered the tube ipsilateral to the dominant follicle, in patients with endometriosis the macrospheres preferentially entered the tube contralateral to the dominant follicle. The difference in ascension into the contralateral tube between the two groups of patients was significant (P<0.01) (from Leyendecker et al., 1996).

Percent of total counts per minute

healthy patients n= 10

comp.1
comp.2
comp.3 ipsilat.
comp.3 contralat.

patients with endometriosis n=14

1   16   32   minutes after application
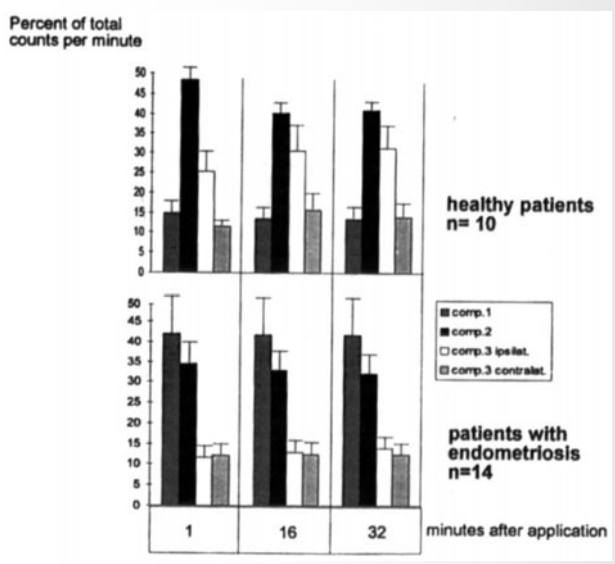
by the dominant follicle in that the uterine myometrium with its specific architecture (Go-erttler, 1930) is activated and contracts in a manner providing this directed transport. In order to elucidate the mechanisms that govern directed sperm ascension, the estrogen re-ceptor distribution within the myometrium was studied. It could be demonstrated in re-moved uteri that the percentage of estrogen receptor positive nuclei of smooth muscle cells in the myometrium was significantly higher on the side of the dominant ovarian structure in comparison with the contralateral side (Kunz et al., 1996b).

The strong correlation between receptor distribution and the site of the dominant ovarian structure suggested that the side-specific asymmetric estrogen receptor distribu-tion is induced by the dominant follicle. This preferential induction of estrogen receptors in the myometrium on the side of the dominant ovarian structure is presumably not ef-fected by the oestradiol concentration in the systemic circulation. Rather, it may involve a more direct endocrine influence mediated, for example, by the utero-ovarian vascular counter-current system (Einer-Jensen, 1988) that may provide higher oestradiol concentra-tions in the uterus on the side of the dominant follicle. This finding may also indicate that other hormone receptors in the uterus, pertinent to its contractile function, might be ex-pressed asymmetrically with respect to the localization of the dominant ovarian structure. Preliminary data show that oxytocin receptors, exhibiting high concentrations in cervical tissue, like those for oestradiol, are asymmetrically distributed within the fundal part of the myometrium relative to the side of the dominant follicle (unpublished).

The asymmetric estrogen receptor distribution being presumably responsible for the directed sperm transport appears to be superimposed on a basal, more evenly distributed level of estrogen receptors under the influence of systemic oestradiol. This may be derived from the observation that the systemic administration of exogenous estrogen to hypogo-nadal women results in a uterine peristaltic activity which is, according to VSUP, indistin-guishable from normal

5. UTERINE HYPERPERISTALSIS AND DYSPERISTALSIS

In studying infertile women suffering from mostly mild endometriosis, which is con-sidered not to be a cause of infertility (Hull et al., 1986; Adamson and Pasta, 1994), a fun-damental disturbance of uterine peristaltic activity was observed (Leyendecker et al., 1996) (Fig 1). In VSUP, these women displayed a considerable degree of hyperperistalsis in that, during the early and mid follicular as well as mid-luteal phase of the cycle, respec-tively, the frequency of peristaltic contraction was nearly doubled in comparison to nor-mal. During the late follicular phase the frequency was increased further but less pronounced as compared to the early and mid-follicular phase of the cycle. The character of the peristaltic activity, however, had completely changed. While in the fertile controls long and regular cervico-fundal peristaltic waves prevailed, the contractions displayed a convulsive character in the infertile women. Some of the contraction waves started in the middle portion of the uterine cavity, while in other patients the contractions started at dif-ferent sites at the same time, and some vanished before reaching the fundal part of the uterine cavity. Thus, in comparison with the regular and frequent cervico-fundal contrac-tions of healthy women, the impression of a dysperistalsis prevailed in patients with infer-tility and endometriosis (Leyendecker and Kunz, 1996).

These abnormalities of the uterine peristaltic activity in women with endometriosis had a profound impact on the uterine transport function as demonstrated by HSSG, which, at least in part, may account for the infertility of these women. Already during the early

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follicular phase of the cycle, there was a rapid transport of inert particles through the uterine cavity into the tubes. This was further increased during the mid follicular phase. However, there was no directed transport into the tube ipsilateral to the dominant follicle. During the late follicular phase, when the uterine contractions had become dysperistaltic, a breakdown of the uterine transport function occurred in that most of the particles remained at the site of application and only a few entered the tubes without a preference for the "dominant" one (Fig. 2–4).

6. IMPLICATIONS REGARDING THE FUNCTION OF THE CERVICAL MUCUS

These data are also pertinent in reconsidering some of the functions of the cervical mucus with regard to sperm ascension.. It is generally assumed that the sperm actively penetrate the cervical mucus and that the scant and viscous cervical mucus of the early follicular phase acts as a barrier in this respect (Moghissi, 1977). The HSSG demonstrates that already in the early follicular phase, in the presence of scant cervical mucus with little spinnbarkeit, a rapid transport of inert particles through the cervical canal occurs (Fig 2). Moreover, the distribution pattern of the labeled macrospheres within the genital tract of women with endometriosis and hyperperistalsis in the early follicular phase (Fig. 2; lower panel) resembles that of the healthy controls with normoperistalsis in the mid follicular phase of the cycle (Fig. 3; upper panel). Thus, it is not so much the quality of the cervical mucus but rather the power of the uterine peristalsis that determines the amount of sperm ascension through the cervical canal.

According to in vitro studies sperm penetrate the cervical mucus at a speed of 0.1 to 3 mm/min depending upon the phase of the cycle. There is no doubt on the basis of our studies that the sperm's own velocity is of little importance with respect to the ascension through the cervical canal. Irrespective of whether there is a function at all to the sperm's active movement at this stage of reproduction, the interaction of the sperm with the physico-chemical properties of the mucus enable viable sperm to enter the cervical crypts as a primary reservoir for later release. Of course, our model using inert albumin macrospheres cannot account for effects that have to be attributed to the functional capacity of healthy sperm.

7. AN INTEGRAL VIEW ON SPERM ASCENSION WITHIN THE FEMALE GENITAL TRACT AND ITS POSSIBLE DISTURBANCES

The clinician has been familiar for a long time with rhythmical contractions of the non-pregnant uterus. Upon cervical inspection during the preovulatory phase rhythmical protrusions of the abundant cervical mucus can be observed. Only recently, due to high resolution ultrasound examination, it was possible to relate these cervical activities to uterine peristaltic waves that originate in the cervix and are propagated towards the cornual section of the uterus. With the placement of labeled inert particles of sperm size at the external cervical os and following their path through the female genital tract by HSSG it was possible to demonstrate the enormous power and transport capacity of this uterine peristaltic pump. Furthermore, the directed transport of the particles preferentially into the

tube ipsilateral to the dominant follicle demonstrated the surprising sophistication of this uterine system of sperm transport (Kunz et al., 1996a).

On the basis of the data obtained in our studies and available from literature, we hypothesize that rapid as well as sustained sperm is controlled by uterine peristaltic activity. Uterine contractions aspirate sperm into the cervical mucus and the uterine cavity, and provide further transport into the isthmical part of the tubes. In the mid- and late follicular phases of the cycle this transport is directed preferentially into the tube ipsilateral to the dominant follicle. This indicates that the mechanism of rapid and passive sperm transport is under the endocrine control of the dominant follicle. Some sperm, probably the most motile ones, follow, by their own movement, the filamentous structures of the cervical mucus and enter the cervical crypts as a primary reservoir. This results in a partial sequestration of the sperm increasing the proportion of less motile and immobile sperm that reach the tubes rapidly. This observation has probably lead to the notion that rapid sperm ascension might not be essential for fertilization (Mortimer, 1983; Hunter, 1987). With the progression of the follicular phase there is an increasing release of sperm from the primary reservoir as they are flushed and squeezed out of the crypts due to the cervical secretion which becomes more profuse and the rhythmical contractions that originate within the cervix, respectively. Entering the "main stream" of cervical secretion they are caught by the "uterine peristaltic system" and rapidly transported in an aliquot of mucus (Fukuda and Fukuda, 1994), which protects the sperm from leukocyte degradation within the uterine cavity (Harper, 1994), to the tube with its isthmic mucus as the secondary reservoir. Dilatation of the external cervical os, maximum cervical secretion and rhythmical protrusion of the mucus around ovulation enlarge the zone of contact between a fresh ejaculate and the uterine peristaltic pump. At the same time preovulatory mucorrhea, together with the rhythmical contractions of the cervix, prevents by large motile sperm from entering the cervical crypts and thus ensures, in combination with maximally increased uterine peristalsis, that no or only minor sequestration of sperm can occur and that motile sperm are directly transported into the isthmical mucus (Jansen, 1980) of the tube ipsilateral to the dominant follicle where they are available for fertilization.

There is, in our opinion, no principle difference between the mechanisms of rapid and sustained sperm transport, respectively. Both aim at the availability of viable sperm at the site of fertilization around ovulation and both rely, in this respect, on the continuous peristaltic activity of the uterus. Sustained sperm transport utilizes the cervical crypts as a primary reservoir, from where later release occurs. The reduced power of the peristaltic pump several days prior to ovulation in comparison to the preovulatory phase might, together with a more viscous cervical mucus at this time, facilitate the migration of motile sperm into the cervical crypts. No data are available, which of the two reservoirs, the cervical or the tubal mucus, have a preponderance in the function of sperm preservation. If there is any preponderance at all, one may assume that it may shift from the cervix to the tube with the progression of the preovulatory phase. In any event, the fundamental importance of sperm preservation within the genital tract and sustained sperm transport for the overall process of reproduction is documented by the observation that intercourse several days prior to ovulation may result in a pregnancy with a considerable probability ranging from about 10% with intercourse five to more than 30% with intercourse two days prior to ovulation, respectively (Wilcox et al., 1995).

These data and considerations show that the availability of sperm at the site of fertilization at the appropriate time depends to a large extent on coordinated uterine peristaltic contractions that cyclically change in quality and frequency. At a low frequency and power, they may favor sperm preservation within the cervical mucus, at a higher preovula-

tory frequency and power of contractions, the uterine peristaltic pump provides rapid and directed transport of sperm either from the reservoir or from a freshly deposited ejaculate into the tube ipsilateral to the dominant follicle.

Recently, it could be shown that this fine-tuned system is fundamentally disturbed in women with infertility and, mostly, mild endometriosis. Both, the hyper- and dysperistalsis of the uterine peristaltic pump observed in these women may contribute to their reduced fertility. Hyperperistalsis may prevent the development of an adequate pool of preserved sperm within the reservoir of the cervical crypts, and preovulatory dysperistalsis impedes, by a breakdown of sperm transport, the formation of an adequate sperm reservoir in the mucus of the isthmical part of the tube from where sperm migrate to the final site of fertilization.

Independent of its effects on sperm transport and fertility uterine hyperperistalsis may promote the detachment and exfoliation of endometrial cells and tissue fragments and their transtubal transport into the peritoneal cavity and may, therefore, propagate the development of endometriosis (Leyendecker et al., 1996).

8. CONCLUSIONS

Uterine peristalsis is of fundamental importance in the process of reproduction in that it serves sperm transport from the external os of the cervix to the mucus of the isthmical part of the tube ipsilateral to the dominant follicle. This mechanism is controlled by the dominant follicle. This newly disclosed and described uterine function is of clinical importance in that a dysfunction of this functional system may result in infertility and may propagate the development of endometriosis.

9. REFERENCES

Abramovicz JS & Archer DF 1990 Uterine endometrial peristalsis - a transvaginal ultrasound study. Fertility and Sterility 54:451–454

Adamson GD & Pasta DJ 1994 Surgical treatment of endometriosis-associated infertility: Meta analysis compared with survival analysis. American Journal of Obstetrics and Gynecology 171: 1488–1505

Amso NN, Crow J & Shaw RW 1994a Comparative immunohistochemical study of oestrogen and progesterone receptors in the fallopian tube and uterus at different stages of the menstrual cycle and the menopause. Human Reproduction 9: 1027 - 1037

Amso NN, Crow J, Lewin J & Shaw RW 1994b A comparative morphological and ultrastructural study of endometrial gland and fallopian tube epithelia at different stages of the menstrual cycle and the menopause. Human Reproduction 9: 2234 - 2241

Becker W, Steck T, Alber P & Borne W 1988 Hystero-salpingo-scinitraphy: A simple and accurate method of evulating fallopian tube patency. Nuklearmedizin 27: 252–257

Birnholz J. (1984) Ultrasonoc visualization of endometrial movements. Fertility and Sterility 41:157–158

Cibils L.A. (1967) Contractility of the non-pregnant human uterus. Obstetrics and Gynecology. 30: 441 - 461

Crow J, Amso NN, Lewin J & Shaw RW 1994 Morphology and ultrastructure of fallopian tube epithelium at different stages of the menstrual cycle and the menopause. Human Reproduction 9: 2224 - 2233

De Vries K, Lyons EA, Ballard G, Levi CS & Lindsay DJ 1990 Contractions of the inner third of the myometrium. Amercan Journal of Obstetrics and Gynecology 162:679–682

Drobnis EZ & Overstreet JW 1992 Natural history of mammalian spermatozoa in the female reproductive tract. Oxford Review of Reproductive Biology 14: 1–46

Einer-Jensen N 1988. Countercurrent transfer in the ovarian pedicle and it's physiological implications. Oxford Reviews of Reproductive Biology 10:348–381

Eliasson R & Posse N 1960 The effect of prostaglandins on the nonpregnant uterus in vivo. Acta Obstetrica et Gynecologica Scandinavica 39: 112–117

Fuchs A-R, Fuchs F & Soloff M.S 1985 Oxytocin receptors in nonpregnant human uterus. Journal of clinical Endocrinology and Metabolism 60: 37–41

Fukuda M & Fukuda K 1994 Uterine endometrial cavity movement and cervical mucus. Human Reproduction 9:1013–1016

Goerttler K 1930 Die Architektur der Muskelwand des menschlichen Uterus und ihre funktionelle Bedeutung. Gegenbaurs Morphologisches Jahrbuch 65:45–52

Harper MJK 1994 Gamete and zygote transport. p123–187 In The physiology of reproduction. Eds Knobil E. & Neill, JD Raven Press, New York,

Hartman CG 1962 Science and the safe period. A compendium of human reproduction. Williams and Wilkins, Baltimore

Hein PR, Eskes TKAB, Stolte LAM, Braaksma JF, Jansens J & Hoek JM 1973 The influence of steroids on uterine motility in the nonpregnant human uterus. p107–140 In Uterine contractions - side effects of steroidal contraceptives. Ed Josimovich, J.B. New York: Wiley and Sons

Hendricks CH 1966 Inherent motility patterns and response characteristics in the nonpregnant uterus. Amercan Journal of Obstetrics and Gynecology 96: 824–841

Hull ME, Moghissi KS, Magyar DF, & Hayes MF 1986 Comparison of different treatment modalities of endometriosis in infertile women. Fertility and Sterility 47: 40–44

Hunter RHF 1987 Human fertilization in vivo with special reference to progression, storage and release of competent spermatozoa. Human Reproduction 2: 329–332

Iturralde M & Venter PP 1981 Hysterosalpingo-radionuclide scintigraphy. Seminars in Nuclear Medicine 11: 301 - 314

Jansen RPS 1980 Cyclic changes in the human fallopian tube isthmus and their functional importance. American Journal of Obstetrics and Gynecology 136: 292–308

Karim SMM & Hillier K 1973 The role of prostaglandins in myometrial contraction. p141–169 In Uterine contraction - side effects of steroidal contraceptives. Ed Josimovich JB New York: Wiley and Sons

Kunz G & Leyendecker G 1996 Uterine peristalsis throughout the menstrual cycle. Physiological and pathophysiological aspects. Human Reproduction update (in press)

Kunz G, Beil D, Deininger H, Wildt L & Leyendecker G 1996a The dynamics of rapid sperm transport through the female genital tract. Evidence from vaginal sonography of uterine peristalsis and hysterosalpingoscintigraphy Human Reproduction 11: 627–632

Kunz G. Mall G & Leyendecker G 1996b Asymmetry of oestrogen receptor expression in the myometrium relative to the site of the dominant follicle and corpus luteum during the menstrual cycle in healthy women. Human Reproduction 11: Abstract book 1, p 158

Leyendecker G, Kunz G, Wildt L, Beil D & Deiniger H 1996 Uterine hyperperistalsis and dysperistalsis as dysfunctions of the mechanism of rapid sperm transport in patients with endometriosis and infertility. Human Reproduction, 11: 1542–1551

Lefebvre DL, Farookhi R, Larcher A, Neculcea J & Zingg HH 1994a Uterine oxytocin gene expression. I. Induction during pseudopregnancy and the estrous cycle. Endocrinology 134: 2556–2561

Lefebvre DL, Farookhi R, Giaid A, Neculcea J & Zingg HH 1994b Uterine oxytocin gene expression. II. Induction by exogenous steroid administration. Endocrinology 134: 2562–2566

Lyons EA, Taylor PJ, Zheng, XH, Ballard G, Levi CS & Kredentser JV 1991 Characterization of subendometrial myometrial contractions throughout the menstrual cycle in normal fertile women. Fertility and Sterility 55:771–775

Martinez-Gaudio M, Yoshida T & Bentsson LP 1973 Propagated and non propgated myometrial contractions in normal menstrual cycles. American Journal of Obstetrics and Gynecology 115: 107–111

Moghissi KS 1977 Sperm migration through the human cervix. In The uterine cervix in reproduction p146–165. Eds Insler V & Bettendorf G. Georg Thieme Publishers Stuttgart

Mortimer D 1983 Sperm transport in the human female reproductive tract. Oxford Reviews of Reproductive Biology 5: 40–61

Oike K, Obata S, Tagaki K, Matsuo K, Ishihara K & Kikuchi S 1988 Observation of endometrial movement with transvaginal sonography. Journal of Ultrasound in Medicine 7: 99

Romanini C 1994 Measurement of uterine contractions. P337–355 In The uterus. Eds Chard J & Grudzinskas JG Cambridge Reviews in Reproduction, Cambridge University Press

Williams M, Hill C.J, Scudamore I, Dunphy B, Cooke ID & Barratt CLR 1993 Sperm numbers and distribution within the human fallopian tube around ovulation. Human Reproduction 8: 2019–2026

Wilcox AJ, Weinberg CR & Baird DD 1995 Timing of sexual intercourse in relation to ovulation - effects on the probability of conception, survival of the pregnancy, and sex of the baby. New England Journal of Medicine 333: 1517–1521

Exhibit 72

Physiology of Upward Transport in the Human Female Genital Tract

I. ZERVOMANOLAKIS,[a] H.W. OTT,[a] D. HADZIOMEROVIC,[a] V. MATTLE,[a]
B. E. SEEBER,[a] I. VIRGOLINI,[b] D. HEUTE,[b] S. KISSLER,[c]
G. LEYENDECKER,[d] AND L. WILDT[a]

[a]*Division of Gynecological Endocrinology and Reproductive Medicine,
Department of Obstetrics and Gynecology, University of Innsbruck, Innsbruck,
Austria*

[b]*Department of Nuclear Medicine, University of Innsbruck, Innsbruck, Austria*

[c]*Department of Obstetrics and Gynecology, University of Frankfurt, Frankfurt,
Germany*

[d]*Department of Obstetrics and Gynecology, Klinikum Darmstadt, Academic
Teaching Hospital to the University of Frankfurt, Darmstadt, Germany*

**ABSTRACT: The uterus and fallopian tubes represent a functionally
united peristaltic pump under the endocrine control of ipsilateral ovary.
We have examined this function by using hysterosalpingoscintigraphy
(HSS), recording of intrauterine pressure, electrohysterography, and
Doppler sonography of the fallopian tubes. An uptake of labeled parti-
cles into the uterus was observed during the follicular and luteal phases
of the cycle after application into the vagina. Transport into the oviducts,
however, could only be demonstrated during the follicular phase. Fur-
thermore, the predominant transport was into the tube ipsilateral to
the ovary containing the dominant follicle. The pregnancy rate following
spontaneous intercourse or insemination was higher in those women in
whom ipsilateral transport could be demonstrated. The amount of ma-
terial transported to the ipsilateral tube was increased after oxytocin ad-
ministration, as demonstrated by radionuclide imaging and by Doppler
sonography following instillation of ultrasound contrast medium. An in-
crease in the basal tone and amplitude of contractions was observed after
oxytocin administration. These results support the idea that the uterus
and fallopian tubes act as a peristaltic pump, which increases transport
of sperm into the oviduct ipsilateral to the ovary bearing the dominant
follicle. Oxytocin appears to play a critical role in this peristaltic pump.
A failure of the peristaltic mechanism is possibly responsible for infertil-
ity. We propose the term tubal transport disorder (TTD) as a nosological
entity. Results from HSS could be a useful adjunct for choosing treat-
ment modalities in patients with patent fallopian tubes suffering from**

Address for correspondence: Prof. Dr. L. Wildt, Clinical Division of Gynaecological Endocrinology
and Reproductive Medicine, Medical University of Innsbruck, Anichstrasse 35, A 6020 Innsbruck,
Austria. Voice: 0043-512-504-23276; fax: 0043-512-504-23277.
  ludwig.wildt@i-med.ac.at

infertility. These patients may be better served with *in vitro* fertilization (IVF).

INTRODUCTION

One of the critical steps in the process of reproduction is the transport of spermatozoa from the vagina to the pars ampullaris of the fallopian tube. Its successful completion requires a mechanical patency as well as a functional integrity of the uterus and the oviducts.[1,2] The biological significance and pathophysiology of transport function, in contrast to simple mechanical patency of the female genital tract, have also not been completely understood. The procedures used for clinical evaluation of the uterus and oviduct, that is, hysterosalpingography (HSG), hysterosalpingocontrastultrasonography (HyCoSy), and laparoscopy with chromopertubation (LSCP) are based on the infusion of liquid media into the uterus, such as a radiological water-soluble contrast medium, a contrast medium developed for ultrasonography and a solution of methylene-blue during laparoscopy, using pressure to force their passage through the fallopian tubes into the abdominal cavity, and mainly assess mechanical patency.[3–5] Mechanical patency does not necessarily equate with functional integrity. Up to now, development and clinical application of methods for the assessment of functional aspects of the genital tract with regard to transport processes have not been available and thus not examined as possible diagnostic tools. By studying aspects of transport mechanisms in the uterus and the fallopian tubes through the use of hysterosalpingoscintigraphy (HSS), in conjunction with other biophysical and pharmacological interventions, we offer new insights into the pathophysiology of the female reproductive tract. Consequently, we suggest that tubal transport disorders (TTD), which can be diagnosed by HSS, may represent a thus far unrecognized factor in infertility.

METHODS

Patients

We reviewed the results of HSS in more than 1,000 women, ages 20–46, suffering from primary or secondary infertility of various etiologies, who underwent HSS followed by HyCoSy, to evaluate uterine and fallopian tube function. These women also underwent other diagnostic procedures, such as cycle monitoring combining determination of luteinizing hormone (LH), estradiol (E2), and progesterone in serum with sonographic determination of follicular development. Informed consent was obtained from all participants.

Materials

Urinary silastic catheters 6 or 8 charriere for intrauterine application of con-
trast medium fitted with an inflatable balloon were used (Uromed Kurt Drews
GmbH, Oststeinbeck, Germany). We prepared the recording electrodes for
electrohysterography using cephalic electrodes from Hewlett Packard Med-
ical Products Group, Waltham MA, USA. Silastic, polyethylene, and teflon
tubing was obtained from Reichelt Chemietechnik, Heidelberg, Germany.

Cycle Monitoring

We defined a dominant follicle as a follicle with a diameter of more than
10 mm and determined LH, E2, and progesterone in blood samples collected
once daily starting on day 10 of the cycle using commercially available im-
munoassays (Boehringer Mannheim, Germany). The luteal phase was assessed
by progesterone levels taken every 2–5 days during the 2 weeks after the be-
ginning of the LH surge until the onset of menstruation. Ultrasonography
was used to monitor follicular development using Siemens Sonoline AC and
Siemens Versa Pro ultrasound devices (Siemens AG, Erlangen, Germany),
both equipped with 5.0–7.5 MHz vaginal probes.

HSS

HSS was performed in the follicular phase of the cycle in 1,000 patients.
Fifteen patients were examined inadvertently during the early- to midluteal
phase. The largest follicle was identified by ultrasonography on the day of
examination and its localization (left or right ovary) and diameter were deter-
mined. Using a catheter we applied 10 ± 2 MBq-TC-99 m labeled macroag-
gregates of human serum albumin (SolcoMAA, Solco Basel AG, Birsfelden
Switzerland) with a size of 5–20 Hm, corresponding roughly to the size of
spermatozoa, in a volume of 1–2 mL to the posterior vaginal fornix with the
patient in a supine position. Scans with a gamma camera were obtained imme-
diately after application and at various time intervals for up to 4 h, as already
described.[6,7] Color printouts of the scans were used for evaluation. A small
mark was set on the skin between symphysis pubis and the umbilicus for topo-
graphical identification. The results were rated as (a) radioactivity within the
cavum uteri, (b) radioactivity within the fallopian tubes, and (c) radioactivity
within the abdominal cavity. Combining the examination with the findings
obtained by ultrasonography, the results were further classified as ipsilateral
when radioactivity concentrated predominantly within the fallopian tube on
the side of the dominant follicle, as contralateral when radioactivity was de-
tected predominantly in the tube opposite to the side of the dominant follicle,

as bilateral when activity was found equally distributed within both tubes, and as unilateral when activity was found within one tube only, but no dominant follicle was identified by ultrasound.

Validation of HSS

A bladder catheter was placed into the uterus in 4 patients after the examination was completed and flushed with 3 mL of saline to ascertain that the labeled material had remained in an intrauterine or intratubal position. The amount of radioactivity in the region of the uterus was determined and compared to that in the abdominal cavity by taking an additional scan after flushing. In addition, fluid was collected from the pouch of Douglas in 3 patients who underwent laparoscopy on the HSS day and the radioactivity of the fluid was counted in a well-type gamma counter. The fluid was divided into two aliquots. One aliquot (0.5 mL) was mixed with 3 mL 20% trichloracetic acid. The sample was centrifuged, the supernatant removed, and the radioactivity in the precipitate was counted. The second aliquot was centrifuged, the pellet washed once with saline and counted after recentrifugation for 10 min. Microscopic examination of the pellet was done.

Effects of Oxytocin

The transport of radiolabeled microspheres was used to examine the effects of oxytocin in 50 patients, using serial HSS scans. The first scan was performed immediately after application of the microspheres to the vagina, followed 8–10 min later by a second scan. After intravenous (i.v.) administration of 3 international units (IU) oxytocin (Syntocinon, Sandoz AG, Nürnberg, Germany), two additional scans were taken, one immediately after oxytocin injection and the second scan 8–10 min later. Regions of interest (ROI) were placed for quantitative evaluation on both sides of the uterus in the area of the fallopian tubes and the radioactivity per unit time within these areas was recorded and plotted.

Measurement of Intrauterine Pressure (Hysterotonography)

During the follicular phase of the cycle, the intrauterine pressure was recorded in 25 patients using either a catheter fitted with two Millar-microtip transducers positioned 6 cm apart or with two catheters filled with sterile water, each connected to a Gould–Statham element as pressure recorder. The catheters were made from teflon or polypropylene tubing with an outer diameter of 1 mm and fitted at the tip with a small, inflatable rubber balloon as pressure

sensor (Hugo Sachs Elektronik, March-Hugstetten, Germany). The catheters
were placed under ultrasonographic guidance into the uterus with the tip of the
first catheter at the fundus (position I), and the tip of the second catheter just
behind the internal os (position II). The catheters were either connected with
an 8-charriere bladder catheter that could be blocked by an inflatable balloon
or held in place by a wire clip attached to the cervix, so that their expulsion
could be avoided. Using this multichannel recorder, the differential between
the pressure measured at positions I and II (intrauterine pressure) was recorded
for 10 to 20 min. Oxytocin was administered either i.v. (3 IU) or as nasal spray
(4 IU) and recording continued for another 10 to 20 min. Frequency and am-
plitude of contractions and the pressure gradients between fundus and cervix
uteri were determined using calipers.

Electrohysterography

Two silver electrodes made from a cephalic electrode were used for recording
uterine electrical activity in 20 patients. The wires were immersed repeatedly
into a solution prepared by mixing 1 mL medical grade silastic adhesive with
5 mL n-Hexane and allowed to dry at room temperature under a light stream
of air for insulation. The insulating silastic layer was then removed carefully
with a scalpel at a length of 2 mm from the tip of the wires. By placing one
electrode into the fundus uteri and fixing the second one at the external os
or within the cervix, we could measure electrical potentials continuously and
record them with a Biofeedback system (SOM Biofeedback, Murrhardt, Ger-
many) connected to a computer. A computer program adapted from a program
for detection of pulses of hormones in plasma[8] identified the amplitude and
frequency of spikes and calculated the variability from point to point.

Doppler Ultrasonography of the Fallopian Tubes

Performing Doppler ultrasonography in 60 patients who underwent HyCoSy
was used to determine flow through the fallopian tubes. We infused contrast
medium (Echovist 300, Schering AG, Berlin, Germany) into the uterus via a
catheter until the uterine cavity and the fallopian tubes could be visualized ei-
ther by vaginal or by abdominal ultrasonography. After removal of the catheter,
a pulsed Doppler beam was directed to the cavum uteri and the fallopian tubes.
The ultrasound probe was held in place by a clamp fitted to a colposcope
holder. Oxytocin was administered i.v. or intranasally at doses of 4 and 3 IU
per application, respectively, after 2–5 min and the recording of Doppler sig-
nals was continued. A video printer was used during the recording periods. An
increase to at least 10 cm/sec for a duration of at least 1 sec was defined as a
signal. Frequency and intensity of the signals on the printout were determined
using mechanical calipers.

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Measurement of Ciliary Beat Frequency

Using a photoelectric technique and fast Fourier transform analysis, we determined the baseline ciliary beat frequency (CBF) of fimbria under standardized temperature conditions. Fimbrial portions of fallopian tubes were collected from 21 patients undergoing post partum sterilization, after obtaining written informed consent and local ethical committee approval. All study subjects had regular menstrual cycles before gravidity and no subject had used hormonal medications during pregnancy. Normal appearing, representative sections of fimbrial tissues, 0.5–1 cm in length, of both fallopian tubes of each subject were rinsed several times to remove all visible evidence of blood. Changes in CBF were documented by ROI measures for temperatures ranging from 37–39°C.

Clinical Evaluation of Tubal Patency

The mechanical tubal patency is defined as the observation of flow into the abdomen revealed by one of the following methods: HSG, HyCoSy, or LSCP. HSG was performed with a Schultze apparatus applied to the cervix for instillation of a radiological water-soluble contrast medium into the uterus (Isovist 300, Schering AG, Berlin, Germany). During HyCoSy a bladder catheter (Kinder- Ballon-Katheter 6 charriere, Uromed) was placed into the uterus and blocked; 2–4 mL of contrast medium developed for ultrasonography (Echovist 300, Schering AG, Berlin, Germany) was infused via the catheter into the uterus. The flow into the uterine cavity and the fallopian tubes was monitored by vaginal ultrasonography. Chromopertubation during laparoscopy was performed by placing a portio adapter into the uterus and infusing a solution of methylene-blue, with visualization of contrast escaping the fimbria as evidence of patency.

Statistical Analysis

The SPSS software package versions 6.1.3–11.00 were used for statistical analysis. Multiple data sets were analyzed by analysis of variance (ANOVA) followed by the Newman–Keuls test for comparing means. The level of significance was set as $P \leq 0.05$. Chi-squared analysis was used for testing distributions. Paired *t*-test was used when the effects of treatment were compared.[9]

RESULTS

Rapid transport of the microspheres from the vagina into the uterine cavity was confirmed by the detection of labeled particles in the uterus at the time of

the first HSS scan, as early as 2 min after intravaginal application. Uptake into the uterus was observed during the follicular as well as during the luteal phases of the cycle in every patient examined. Radioactivity entered the fallopian tubes either on both sides (15%) or on only one side (64%) in 79% of the patients studied during the follicular phase. In the remaining patients, radioactivity was detected only in the uterine cavity and did not migrate into the fallopian tubes. FIGURE 1 shows typical examples of the scans. Significant radioactivity entering the pelvis was observed in only 6% of the patients.

The ascension of radioactive particles into the uterus in the 15 patients examined during the luteal phase appeared to be indistinguishable from that observed during the follicular phase. However, we did not observe transport into the oviducts in any of the patients examined during the luteal phase. In addition, in these 10 women, we observed a qualitatively different pattern of distribution of radioactivity within the uterus compared to that observed during the follicular phase of the cycle. A rather broad area of radioactivity was observed during the luteal phase giving the impression of a large cavum uteri, while during the follicular phase, the area of maximal activity had an elongated shape.

Radioactivity from the uterine cavity and the oviducts was completely dispersed by flushing the uterus with a small volume of saline ($\sim$ 3 mL). In addition, more than 90% of radioactivity in the fluid collected from the cul de sac in 3 patients who underwent laparoscopy was found in the pellet after centrifugation and could be precipitated by trichloracetic acid, indicating that most of the radioactivity was still protein bound.

FIGURE 2 shows the relationship between ipsilateral and bilateral entry of radioactivity into the fallopian tubes and the size of the dominant follicle. The frequency of ipsilateral transport of activity into the oviduct was found to increase from 10% to 75% with increasing diameter of the leading follicle,
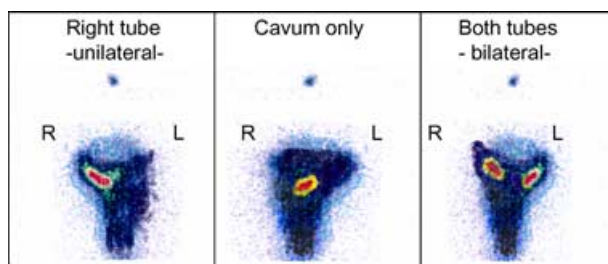

FIGURE 1. Typical examples of scans taken 10–20 min after application of 10–12 MBq 99mTc labeled microspheres to the posterior vaginal fornix, demonstrating (from left to right) uptake into the uterus and unilateral transport to the right fallopian tube (**A**), uptake into the uterus only (**B**), and bilateral transport into the oviducts (**C**). A marker is placed at half distance between the symphysis and umbilicus (reprinted from Wildt *et al*.[27] with permission).
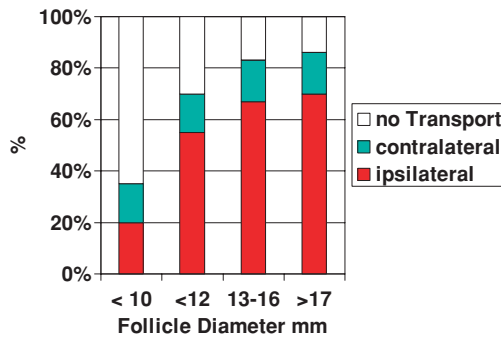
FIGURE 2. Lateralization of transport of labeled microspheres and size of the lead-ing follicle. With increasing diameter of the dominant follicle, the proportion of patients exhibiting ipsilateral transport to the oviduct leading to the dominant follicle did increase progressively. The proportion of patients who had ipsilateral transport was higher in those who became pregnant after timed intercourse or intrauterine insemination than in those who did not conceive after this treatment (Treatment duration lasting up to 6 cycles). Up to a follicle size of 13 mm, ipsilateral transport could be diagnosed only in retrospect, at the time when a dominant follicle appeared on the side where radioactivity was concentrated.

when all patients were included in the analysis. The percentage of patients with ipsilateral transport was higher and increased from 25% to 95%, when only those patients were considered who later became pregnant either spontaneously or after intrauterine insemination.

TABLE 1 shows the relationship between the outcome of treatment of infertil-ity and the asymmetrical distribution of radioactivity. The combined pregnancy rate for spontaneous pregnancies (Sp) or pregnancies following intrauterine insemination (IUI) in women exhibiting ipsilateral transport was 21.7%; when no entry of radioactivity into the tubes was found, the pregnancy rate was only 2% ($P < 0.05$). In contrast, no significant difference in pregnancy rate (22.7% vs. 24.5%, respectively) could be observed between both groups of patients who underwent *in vitro* fertilization (IVF) or intracytoplasmic sperm injection (ICSI).

The effects of oxytocin administration on transport of radioactivity are shown in FIGURES 3 AND 4. After oxytocin administration, radioactivity within

TABLE 1. Relationship between the outcome of treatment for infertility and the symmet-rical distribution of radioactivity

	Ipsilateral Transport	No Transport
Pregnant (Sp*+ IUI)	78/360 (21.7 %)	4/200 (2%)
Pregnant ** (IVF+ICSI)	25/110 (22.7%)	48/196 (24.5%)

*includes pregnancies after normal and timed intercourse.
**includes pregnancies after transfer of cryopreserved pronucleus cells.

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the ROI on the ipsilateral side immediately increased, suggesting an increase in the amount of particles transported as a consequence of the administration of the peptide, as shown in FIGURE 3. Radioactivity on the contralateral side, in contrast, did not exhibit dramatic changes. FIGURE 4 summarizes the data for all 50 patients studied. During the luteal phase, oxytocin had no effect on the distribution of radioactivity within the uterus.

Doppler ultrasonography of the uterus and the oviduct filled with contrast medium resulted in eddy formations, indicative of turbulent rather than laminar flow within the tubes. Oxytocin administration resulted in an increase of turbulent flow, as shown in FIGURE 5, but only within the oviduct on the side of the dominant follicle. Only few signals could be detected on the contralateral side before and after the administration of oxytocin.

FIGURE 6 shows the results of the recording of the intrauterine pressure during the follicular phase of the cycle before and after oxytocin administration. Basal pressure increased significantly ($P < 0.05$) immediately following the administration of oxytocin.

FIGURE 7 shows the results of the recording of the CBF under a constant physiological temperature of $37°C$. The mean ($\pm$ SD) baseline in tubal explants
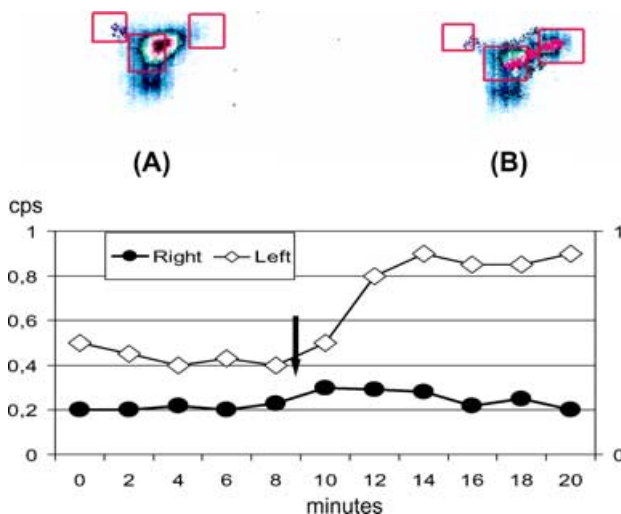

FIGURE 3. Intravenous (i.v.) administration of oxytocin (3 IU) in a patient during HSS. The upper panel (**A** and **B**) shows two scans taken 10 min apart, with the regions of interest (ROI) depicted as boxes over the cavum and the left and right oviducts, respectively. The lower panel shows radioactivity measured within the ROIs over the left and right oviducts and expressed as counts per second. The dominant follicle in this patient was located in the left ovary. Activity on the left side is higher than on the right side. The arrow marks the time when oxytocin was administered; this was followed by an increase of radioactivity found within the ROI on the left side, indicating increased transport into the left oviduct (reprinted from Wildt *et al*.[27] with permission).
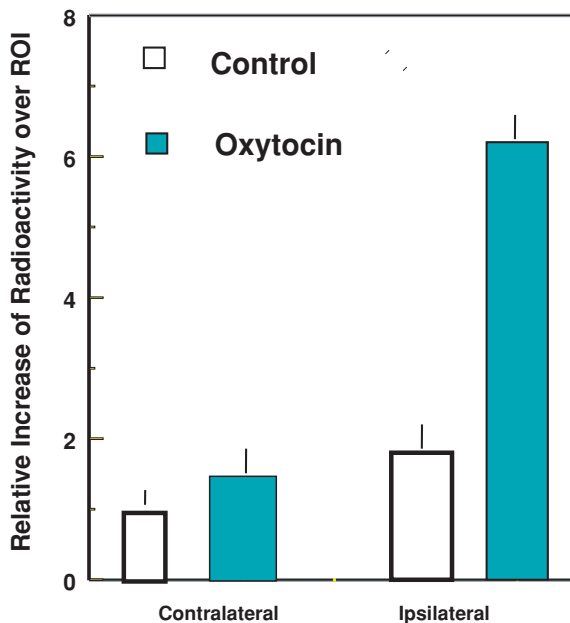
FIGURE 4. Relative increase of the radioactivity detected within the ROI placed over uterus and the fallopian tube leading to the dominant follicle. There is a significant ($P \leq 0.05$) increase in radioactivity immediately after oxytocin administration on the dominant, but not at the contralateral side. Data represent mean $\pm$ SD of 50 observations (reprinted from Wildt et al.[27] with permission).

of the study population was 7.5 ± 0.5 Hz. A significant increase of CBF of 20% (9.5 ± 0.5 Hz) ($P < 0.05$) was recorded after the temperature increased from $37°$C to $39°$C.

DISCUSSION

Male germ cells have to migrate from the posterior vaginal fornix to the pars ampullaris of the fallopian tubes to fertilize an oocyte; the fertilized oocyte then has to be transported to the uterine cavity for implantation. The mechanisms and timing of this bidirectional travel are not completely understood. We studied the migration of radiolabeled immotile aggregates of serum albumin, used as surrogates for spermatozoa, from the vagina through the genital tract and explored some of the factors affecting this migration. We provide evidence that upstream transport in the genital tract may be composed of two components: a rapid uptake by the uterus from the vagina and a directed transport from the uterus to the oviduct toward the ovary bearing the dominant follicle. The former is observed during the follicular and luteal phase of the cycle, while
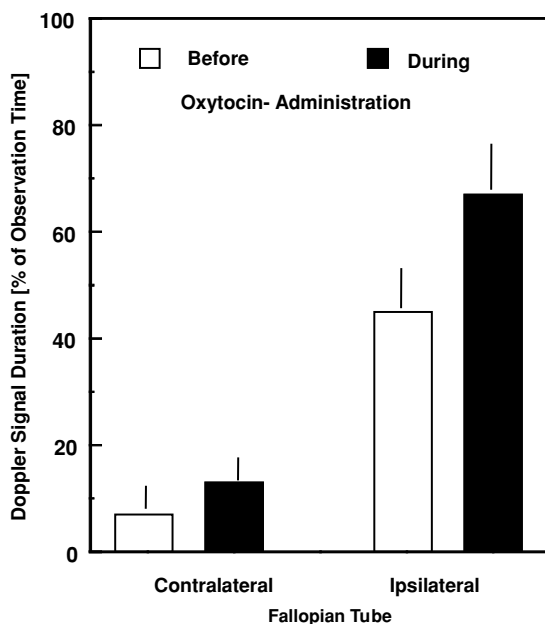
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FIGURE 5. Doppler ultrasonography of the left and right fallopian tubes following instillation of echogenic contrast medium before and after oxytocin administration, demonstrating a significant increase ($P \leq 0.05$) in signal density and frequency after administration of the hormone. Data represent mean $\pm$ SD of 30 observations (reprinted from Wildt *et al*.[27] with permission).

the latter is restricted to the follicular and preovulatory phase, becoming more prominent when the size of the leading follicle increases. Therefore, we believe that the ovary bearing the dominant follicle controls this directed transport.

All examined patients exhibited an uptake of the radiolabeled aggregates by the uterus. This is an indication that this part of the transport mechanism is rather stable and that inhibition of sperm uptake does not represent a major factor in infertility. The observation of this uptake into the uterus during the luteal phase of the cycle was rather unexpected because of the hypothesis that the cervical mucus becomes impenetrable for spermatozoa under the influence of elevated progesterone serum levels. Spermatozoa have previously been shown to be immotile in luteal phase mucus *in vivo* and *in vitro*, resulting in a failure to penetrate cervical mucus *in vitro* experiments.[10–13] Our results may indicate that this does not necessarily affect that passive transport of spermatozoa, which may not be blocked during the luteal phase. Similar numbers of motile spermatozoa are found within the oviduct during the luteal as in the early- to midfollicular phase of the cycle, as previously reported by studies that examined the presence of spermatozoa in different compartments of the genital tract after intercourse. Nevertheless, the highest number of spermatozoa
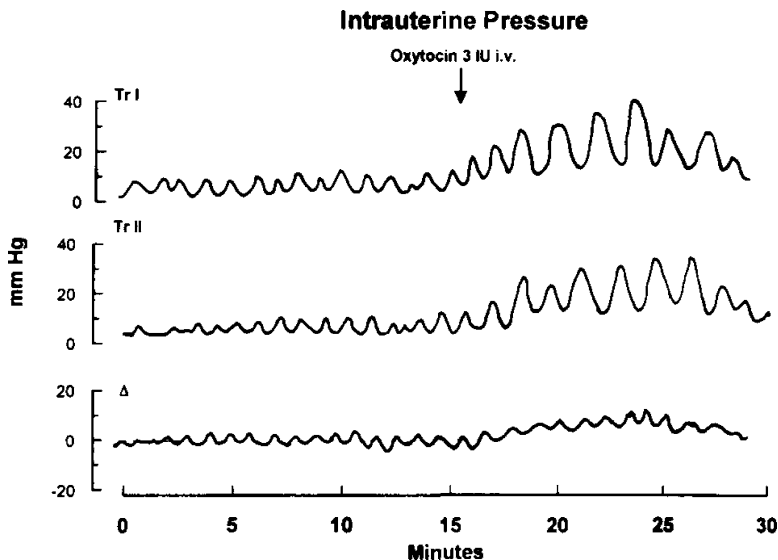
FIGURE 6. Intrauterine pressure recorded during the follicular phase of the cycle before and after the i.v. administration of 3 IU oxytocin. Transducer I (TrI) was placed in the fundal part (**A**), transducer II (TrII) near the internal cervical os (**B**). The arithmetic difference between pressure recorded in position II and the pressure recorded in position I is plotted in the lowest panel (**C**). Note the increase in basal tonus and the increase of the pressure difference between the two recording sites after oxytocin administration. The effect of oxytocin lasted for 20–40 min (reprinted from Wildt *et al*., 1998[27] with permission).

can be detected in the fallopian tube during the preovulatory phase.[10–14] With regard to sperm transport, our interpretation of the results of HSS is based on the assumptions that the properties of the labeled material used for examination are similar to those of human spermatozoa and that there is no separation of label from the carrier *in vivo*. Various radiolabeled compounds have been used the past 30 years for radionuclide imaging of the female genital tract, including aggregates of human albumin, radioactive inert gases, and labeled spermatozoa.[6,15–22] In this study, human serum albumin macroaggregates with Tc-99m attached to the protein by noncovalent binding were used as surrogates for spermatozoa. We feel that we can adequately confirm that the radioactivity was protein-bound because: (1) we observed the disappearance of the radioactivity from the uterus after flushing with saline and (2) the radioactivity collected from the cul de sac at laparoscopy could be precipitated completely by acid and still could be centrifuged down at low speed 4–7 h after application excluding uptake by the lymphatic system.

Although HSS has not been widely used, it is a technically very simple procedure with little discomfort to the patient, in contrast to HSG. There is an apparent discrepancy between the results of HSS and those obtained with HSG
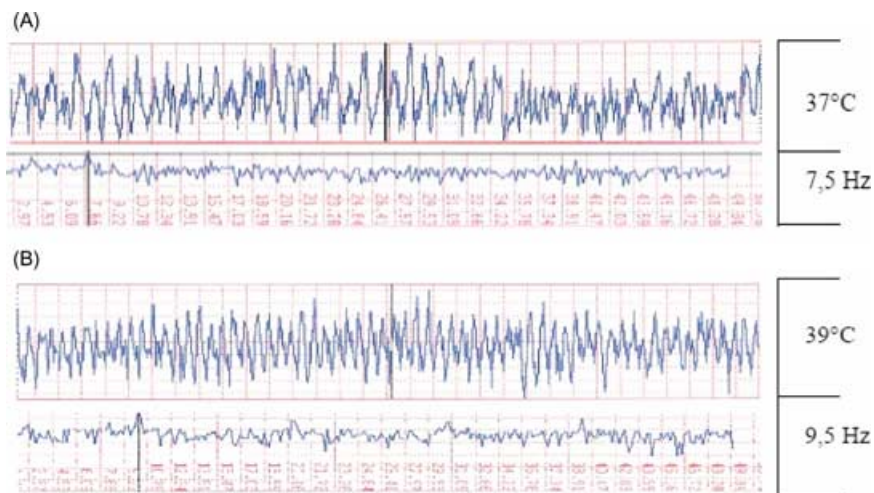
FIGURE 7. Ciliary beat frequency (CBF) is a local phenomenon in fimbrial cilia of human oviducts. Under physiological temperature conditions until 37°C, CBF is 7.5Hz ± 0.5Hz (**A**). As temperature increased to 39°C, CBF increased exponentially up to 20% (9.5 ± 0.5Hz, (**B**). A postovulatory increase of CBF in local areas of fimbrial cilia cells of the ipsilateral tube may guarantee the pickup of the oocyte–cumulus complex into the tube.

or laparoscopy; in the majority of patients with proven patency of both fallopian tubes only one oviduct could be visualized by HSS. The transport of the radiolabeled microspheres is physiologically restricted to the oviduct leading to the ovary bearing the dominant follicle while the contralateral fallopian tube appears to be functionally closed, as it is observed by a detailed analysis of the results of HSS and their correlation with the results of ultrasonography and determination of endocrine parameters for follicular growth.[23–26] Our present study demonstrates that ipsilateral transport, as first shown by our group in 1992,[23] is a reflection of the physiological function of the uterus and the oviduct and not the consequence of tubal pathology or an artifact of the method. This demonstration is supported by the observation that the pregnancy rate after normal intercourse or intrauterine insemination was significantly higher in patients exhibiting ipsilateral transport than in those who did not.

Our results also imply that failure of transport in patients with otherwise mechanically patent fallopian tubes, may be considered an etiology of infertility. Most of the patients examined in this study would have been diagnosed as suffering from idiopathic infertility. We proposed the concept of TTD as a more adequate description of the condition of these patients.[27] The results of HSS may provide criteria for the choice of the adequate therapy in such women, since pregnancy rates in patients with TTD, which are extremely low following insemination or timed intercourse, can be increased substantially by *in vitro* fertilization.

The ovary bearing the dominant follicle appears to control the transport from the uterus to the oviduct. The proportion of patients exhibiting ipsilateral transport increased with the size of the dominant follicle, reaching up to 90% of those patients who became pregnant when the follicle diameter was 19 mm or more. The forces that are driving transport and the mechanisms directing this process need to be defined. Since the particles used for HSS are protein aggregates devoid of motility, motility of the spermatozoa can be excluded. Movements of the ciliae within the oviduct do not seem to be a major factor in rapid transport, since the beat of ciliae is directed from the ampulla to the uterus (the opposite direction), and women with Kartagener Syndrome, that is, congenital absence of ciliae, have no difficulties in becoming pregnant.[1,28–31] It is also unlikely that capillary forces generated within the mucus and a difference in hydrostatic pressure between vagina and peritoneal cavity account for the immediate uptake from the vagina and the directed transport.[32] Peristaltic contractions of the uterus and of the muscular layers of the fallopian tubes therefore represent the most likely candidates responsible for the rapid transport phenomena. Using direct measurement of intrauterine pressure or vaginal ultrasonography combined with videocinematography during the follicular phase of the cycle, peristaltic contractions of the nonpregnant uterus have been described in women during the normal menstrual cycle as well as in women suffering from primary dysmenorrhea or in women with endometriosis.[33–37] The contractions seem to occur with a frequency of 2– 5 per min and to exhibit a characteristic pattern of propagation in healthy women, depending on the phase of the menstrual cycle. While a cervicofundal propagation of peristaltic waves was found during the preovulatory phase of the cycle, a fundocervical direction predominated in the early follicular phase.

The strong positive correlation between the temperature and the oocyte pick-up rate in the animaloviductal infundibulum is demonstrated by a linear regression.[38] Preovulatory temperature differences between the ampullary and isthmic portions of a single tube have been previously reported and thought to primarily reflect the extent and activity of the vascular and lymphatic beds in the oviduct tissues.[39–41] We found periovulatory temperature differences of up to 1.5 °C between the two oviducts measured *in vivo* during tubal catheterization in a small group of patients, temperature being higher within the oviduct leading to the ovary bearing the dominant follicle (Wildt *et al.* unpublished) . Furthermore, we found an exponential increase in CBF in the range of physiological temperature. This is in accordance with the report of a significantly higher temperature in the ipsilateral tube corresponding to the dominant follicle compared to the contralateral side in human oviducts.[42] Our data suggest that this difference in periovulatory temperature between the two fimbriae may be responsible for the increased CBF on the side ipsilateral to the dominant follicle, underlining the concept of the uterus consisting of two functionally different components.

A number of hormones and paracrine mediators, such as prostaglandins, vasopressin, oxytocin, and various peptides can induce uterine contractions.[43–46] The administration of oxytocin during HSS was followed by a five- to sevenfold increase in the radioactivity detected in the oviduct ipsilateral to the dominant follicle; in addition, systemic administration of oxytocin increased the amplitude of contractions and reversed the pressure gradient from a fundocervical to a cervicofundal direction. Oxytocin is known to play an important role in eliciting contractions of the pregnant and nonpregnant uterus, while oxytocin receptors have been demonstrated in the nonpregnant uterus of human females and laboratory animals.[43,47–50] Following vaginal distension and cervical stimulation during intercourse, oxytocin is released from the posterior lobe of the pituitary in response to tactile as well as emotional stimuli.[32,51–55] Synthesis of oxytocin has also been demonstrated within the endometrium and the ovary, respectively.[50,56–59] Knaus demonstrated that injections of posterior pituitary extract containing oxytocin promptly induced contractions of the nonpregnant human uterus during the follicular phase, but not after ovulation.[60] Our results show a striking effect of oxytocin on uterine transport mechanisms and are in agreement with the early observations of Knaus, demonstrating the absence of directed transport during the luteal phase.

The electrical activity as a response to the oxytocin administration corresponded to the increase of intrauterine pressure. In most instances, no direct relationship between contractions and electrical activity was found. Further studies are necessary to explore the correlation between electrical activity and intrauterine pressure and to examine the validity of recording electrical potentials for the assessment of uterine contractions.[61,62] The Doppler ultrasonography after administration of ultrasound contrast medium supports the observation of ipsilateral transport into and within the oviduct during scintigraphy. An increase in turbulent flow within the fallopian tube is indicated by an increase in signal density that was consistently observed immediately after oxytocin administration, either i.v. or intranasally. The increase of flow could only be detected within the oviduct leading to the dominant follicle, which shows that transport occurred predominantly in this direction.[27,63]

Although the overall increase in transport may be explained by the stimulatory action of oxytocin on myometrial contractions, additional mechanisms acting at the levels of uterus and oviduct, such as asymmetric distribution of oxytocin receptors or changes in the resistance of the oviducts caused by the activation or relaxation of smooth muscle cells at the uterotubal junction, are required to account for the unilateral transport. This question cannot be answered by the present studies. Therefore, we propose the following hypothesis, schematically depicted in FIGURE 8: (1) The fallopian tubes are functionally closed in the absence of ovarian hormones. (2) Hormones that cause relaxation of smooth muscle cells are produced by the ovary bearing the dominant follicle. (3) Unilateral transport is to be regarded as the consequence of active relaxation of the myometrium at the side of the ovary that is bearing the
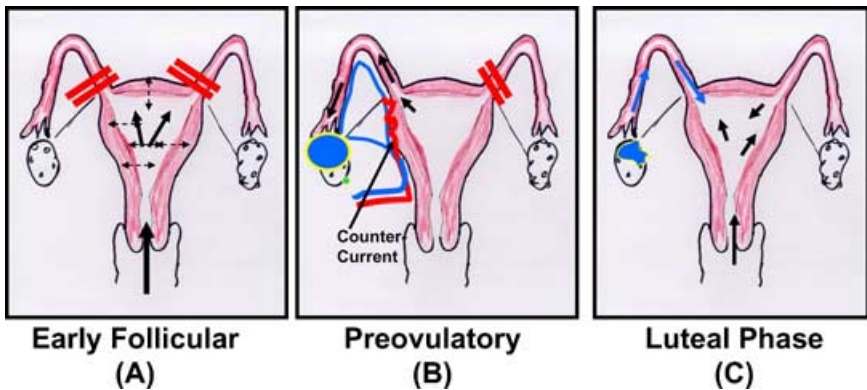
Early Follicular (A) **Preovulatory (B)** **Luteal Phase (C)**

FIGURE 8. Schematic representation of the model of directed transport. During the early follicular phase (**A**) both fallopian tubes are functionally closed, transport occurs from the vagina to the uterine cavity. Contractions of the myometrium, indicated by the broken arrows, which are followed by relaxation, cause a negative pressure within the uterus when compared to the vagina. (**B**) The dominant follicle has been selected. Concentrations of progesterone, produced by the dominant follicle, are elevated at the uterotubal junction due to a countercurrent system indicated by the arrows, causing relaxation of the musculature. Since the contralateral side remains functionally closed, transport is directed into the fallopian tube at the side of the dominant follicle. (**C**) Demonstrates transport during the luteal phase of the cycle. Uptake into the uterus is not impaired, but transport into the fallopian tubes appears to be completely blocked. Transport of the fertilized oocyte is depicted by the arrows within the right fallopian tube, however, the mechanisms governing embryo transport remain to be delucidated.

dominant follicle rather than the induction of a contraction at the contralateral side.

Progesterone can also induce relaxation of the myometrium. The preovulatory follicle produces progesterone in increasing amounts. In addition, progesterone concentrations in the venous effluent from the ovary bearing the dominant follicle are higher than those from the contralateral ovary several days before ovulation.[64,65] Progesterone could be delivered to the area of the uterotubal junction through the arteriovenous countercurrent exchange system that has been identified between the ovary and the uterus.[66–70]

In conclusion, our data demonstrate that the uterus and fallopian tubes seem to act as a functional unit and peristaltic pump that provides the pressure gradients necessary to transport spermatozoa from the vagina to the fallopian tubes. Secretory products originating from the ovary bearing the dominant follicle allow further transport to the ampullary part of the tube on the side of the follicle destined to ovulate, inducing the active relaxation of a functional sphincter mechanism located in the area of the uterotubal junction, while the contralateral oviduct remains functionally closed. Consequently, the probability for fertilization is increased by the maximized number of spermatozoa at

the site where the oocyte is released. Oxytocin contributes to the control of this process by activating pump mechanisms via contraction of uterine smooth muscles. Disturbance of these mechanisms interferes with tubal transport, causing infertility, even in the presence of mechanically open fallopian tubes. HSS appears to be a suitable method to diagnose this TTD.

REFERENCES

1. LYONS, R.A., E. SARIDOGAN & O. DJAHANBAKHCH. 2006. The reproductive significance of human Fallopian tube cilia. Hum. Reprod. Update **12:** 363–372.
2. PULKKINEN, M.O. 1995. Oviductal function is critical for very early human life. Ann. Med. **27:** 307–310.
3. ADELUSI, B., L. AL-NUAIM, D. MAKANJUOLA, *et al.* 1995. Accuracy of hysterosalpingography and laparoscopic hydrotubation in diagnosis of tubal patency. Fertil. Steril. **63:** 1016–1020.
4. KARANDE, V.C., D.E. PRATT., D.S. RABIN, *et al.* 1995. The limited value of hysterosalpingography in assessing tubal status and fertility potential. Fertil. Steril. **63:** 1167–1171.
5. SHAHID, N., A. AHLUWALIA, S. BRIGGS, *et al.* 2005. An audit of patients investigated by hysterosalpingo-contrast-ultrasonography (HyCoSy) for infertility. J. Obstet. Gynaecol. **25:** 275–278.
6. BECKER, W., T. STECK, P. ALBERT, *et al.* 1988. Hysterosalpingoscintigraphy: a simple and accurate method of evaluating fallopian tube patency. Nuklearmedizin. **27:** 252–257.
7. STECK, T., W. BECKER, P. ALBERT, *et al.* 1989. Initial experiences with hysterosalpingoscintigraphy in normal and pathologic fallopian tube passage. Geburtshilfe und Frauenheilkunde. **49:** 889–893.
8. MERRIAM, G.R. & K.W. WACHTER. 1982. Algorithms for the study of episodic hormone secretion. Am. J. Physiol. **243:** 310–318.
9. ZAR, J.H. 1984. Biostatistical Analysis. Second edition. Prentice-Hall. Englewood Cliffs, NJ.
10. BIANCHI, P.G., A. DE AGOSTINI, J. FOURNIER, *et al.* 2004. Human cervical mucus can act *in vitro* as a selective barrier against spermatozoa carrying fragmented DNA and chromatin structural abnormalities. J. Assist. Reprod. Genet. **21:** 97–102.
11. CHRETIEN, F.C., C. SUREAU & C. NEAU. 1980. Experimental study of cervical blockage induced by continuous low-dose oral progestogens. Contraception **22:** 445–456.
12. GADDUM-ROSSE, P., R.J. BLANDAU & W.I. LEE. 1980. Sperm penetration into cervical mucus *in vitro*. II. Human spermatozoa in bovine mucus. Fertil. Steril. **33:** 644–648.
13. MORALES, P., M. ROCO & P. VIGIL. 1993. Human cervical mucus: relationship between biochemical characteristics and ability to allow migration of spermatozoa. Hum. Reprod. **8:** 78–83.
14. VIGIL, P., R. RIQUELME & P. MORALES. 1995. Sperm binding to the human zona pellucida after migration through human cervical mucus. Int. J. Androl. **18:** 7–11.
15. ALIMOV, S.H.A., A.A. KOGAN, V.S. GINZBURG, *et al.* 1973. Radioisotope study of uterine tubal patency. Akush.Ginekol. Mosk. **49:** 64–67.

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16. ARDRAN, G.M. & F.H. KEMP. 1972. Radiography of potentially pregnant females. Br. Med. J. **4:** 422–423.
17. BRUNDIN, J., M. DAHLBORN, E. AHLBERG AHRE, *et al.* 1989. Radionuclide hysterosalpingography for measurement of human oviductal function. Int. J. Gynaecol. Obstet. **28:** 53–59.
18. DE ROSSI, G., M. SALVATORI, A. GIORDANO, *et al.* 1981. Hysterosalpingoscintigraphy. About the possibility of fallopian tubes patency evaluations by means of 99mTc-pertechnetate and comparison of radiation loads due to radioisotopic and x-ray examinations. Radiol. Diagn. Berl. **46:** 692–695.
19. ITURRALDE, M. & P.F. VENTER. 1981. Hysterosalpingo-radionuclide scintigraphy. Semin. Nucl. Med. **11:** 301–314.
20. OZGUR, K., A. YILDIZ, M. UNER., et al. 1997. Radionuclide hysterosalpingography with radiolabeled spermatozoa. Fertil. Steril. **67:** 751–755.
21. PERTYNSKI, T., W. JAKUBOWSKI, J. STELMACHOW, *et al.* 1977. A scintigraphic method of examining the patency of oviducts using 133Xe. Eur. J. Nucl. Med. **2:** 159–164.
22. SCHMIEDEHAUSEN, K., S. KAT, N. ALBERT, *et al.* 2003. Determination of velocity of tubar transport with dynamic hysterosalpingoscintigraphy. Nucl. Med. Commun. **24:** 865–870.
23. BECKER, W., L. WILDT., E. SIEBZEHNRUEBL, *et al.* 1992. Tubal patency regulated by the endocrine system—new concept based on hysterosalpingoszintigraphy. Acta Endocrinologica **126:** 135.
24. KISSLER, S., K. DOINGHAUS, W. BECKER, *et al.* 1995. Hysterosalpingoscintigraphic examination of the fallopian tube: a selective, unilateral transport mechanism. Contracep. Fertil. Steril. **23:** 286.
25. KUNZ, G., D. BEIL, H. DEINIGER, *et al.* 1997. The dynamics of rapid sperm transport through the female genital tract: evidence from vaginal ultrasonography of uterine peristalsis and hysterosalpingoscintigraphy. Hum. Reprod. **11:** 627–632.
26. KUNZ, G., D. BEIL, P. HUPPERT, *et al.* 2006. Control and function of uterine peristalsis during the human luteal phase. Reprod. Biomed. Online **13:** 528–540.
27. WILDT, L., S. KISSLER, P. LICHT, *et al.* 1998. Sperm transport in the human female genital tract and its modulation by oxytocin as assessed by hysterosalpingoscintigraphy, hysterotonography, electrohysterography and Doppler ultrasonography. Hum. Reprod. Update **4:** 655–666.
28. AMSLER, P. & N. PAVIC. 1985. Pregnancy in a patient with immotile cilia syndrome (Kartagener syndrome). Geburtshilfe Frauenheilkd. **45:** 183–185.
29. ELIJAHU, S. & E. SHALEV. 1996. A fertile woman with Kartagener's syndrome and three consecutive pregnancies. Hum. Reprod. **11:** 683.
30. HALBERT, S.A., D.L. PATTON, P.W. ZARUTSKIE, *et al.* 1997. Function and structure of cilia in the fallopian tube of an infertile woman with Kartagener's syndrome. Hum. Reprod. **12:** 55–58.
31. MC COMB, P., L. LANGLEY, M. VILLALON, *et al.* 1986. The oviductal cilia and Kartagener's syndrome. Fertil. Steril. **46:** 412–416.
32. FOX, C.A., H.S. WOLFF & J.A. BAKER. 1970. Measurement of intra-vaginal and intra-uterine pressures during human coitus by radio-telemetry. J. Reprod. Fertil. **22:** 243–255.
33. CSAPO, A.I. & C.R. PINTO DANTAS. 1966. The cyclic activity of the nonpregnant human uterus. A new method for recording intrauterine pressure. Fertil. Steril. **17:** 34–38.

34. DAWOOD, M.Y. 1986. Current concepts in the aetiology and treatment of primary dysmenorrhea. Acta Obstet. Gynecol. Scand. Suppl. **138:** 7–10.
35. KISSLER, S., N. HAMSCHO, S. ZANGOS, *et al.* 2006. Uterotubal transport disorder in adenomyosis and endometriosis—a cause for infertility. BJOG **113:** 902–908.
36. KUNZ, G., D. BEIL, H. DEINIGER, *et al.* 1997. The uterine peristaltic pump. Normal and impeded sperm transport within the female genital tract. Adv. Exp. Med. Biol. **424:** 267–277.
37. LEYENDECKER, G., G. KUNZ, L. WILDT, *et al.* 1996. Uterine hyperperistalsis and dysperistalsis as dysfunctions of the mechanism of rapid sperm transport in patients with endometriosis and infertility. Hum. Reprod. **11:** 1542–1551.
38. HUANG, S., N. DRIESSEN, M. KNOLL, *et al.* 1997. *In vitro* analysis of oocyte cumulus complex pick up rate in the hamster *Mesocricetus auratus*. Mol. Reprod. Dev. **47:** 312–322.
39. HUNTER, R.H. & R. NICHOL. 1986. A preovulatory temperature gradient between the isthmus and ampulla of pig oviducts during the phase of sperm storage. J. Reprod. Fertil. **2:** 599–606.
40. HUNTER, R.H. 2005. The fallopian tubes in domestic mammals: how vital is their physiological activity? Reprod. Nutr. Dev. **45:** 281–290.
41. BAHAT, A., M. EISENBACH & I. TUR-KASPA. 2005. Periovulatory increase in temperature difference within the rabbit oviduct. Human Reprod. **20:** 2118–2121.
42. CICINELLI, E., N. EINER-JENSEN, R. ALFONSO, *et al.* 2005. A dominant ovarian follicle induces unilateral changes in the origin of the blood supply to the tubal corner of the uterus. Hum. Reprod. **20:** 3208–3211.
43. AKERLUND, M. 2006. Targeting the oxytocin receptor to relax the myometrium. Expert Opin. Ther. Targets **10:** 423–427.
44. AKERLUND, M. 2002. Involvement of oxytocin and vasopressin in the pathophysiology of preterm labor and primary dysmenorrhea. Prog. Brain Res. **139:** 359–365.
45. MAGGI, M., E. BALDI & T. SUSINI. 1994. Hormonal and local regulation of uterine activity during parturition: Part I–The oxytocin system. J. Endocrinol. Invest. **17:** 739–756.
46. MAGGI, M., E. BALDI & T. SUSINI. 1994. Hormonal and local regulation of uterine activity during parturition: Part II–The prostaglandin and adrenergic systems. J. Endocrinol. Invest. **17:** 757–770.
47. TERRILLON, S., L.L. CHENG, S. STOEV, *et al.* 2002. Synthesis and characterization of fluorescent antagonists and agonists for human oxytocin and vasopressin V(1)(a) receptors. J. Med. Chem. **45:** 2579–2588.
48. FUCHS, A.R., F. FUCHS & M.S. SOLOFF. 1985. Oxytocin receptors in nonpregnant human uterus. J. Clin. Endocrinol. Metab. **60:** 37–41.
49. MILLER, F.D., R. CHIBBAR & B.F. MITCHELL. 1993. Synthesis of oxytocin in amnion, chorion and decidua: a potential paracrine role for oxytocin in the onset of human parturition. Regul. Pept. **45:** 247–251.
50. IVELL, R., W. RUST, A. EINSPANIER, *et al.* 1995. Oxytocin and oxytocin receptor gene expression in the reproductive tract of the pregnant cow: rescue of luteal oxytocin production at term. Biol. Reprod. **53:** 553–560.
51. FOX, C.A. & B. FOX. 1971. A comparative study of coital physiology, with special reference to the sexual climax. J. Reprod. Fertil. **24:** 319–336.
52. FOX, C.A. 1973. Recent studies in human coital physiology. Clin. Endocrinol. Metab. **2:** 527–543.
53. FOX, C.A. 1976. Some aspects and implications of coital physiology. J. Sex. Marital. Ther. **2:** 205–213.

54. MURPHY, M.R., J.R. SECKL, S. BURTON, *et al.* 1987. Changes in oxytocin and vasopressin secretion during sexual activity in men. J. Clin. Endocrinol. Metab. **65:** 738–741.
55. GILBERT, C.L., K. JENKINS & D.C. WATHES. 1991. Pulsatile release of oxytocin into the circulation of the ewe during oestrus, mating and the early luteal phase. J. Reprod. Fertil. **91:** 337–346.
56. BURNS, P.D., J.O. MENDES, JR., R.S. YEMM, *et al.* 2001. Cellular mechanisms by which oxytocin mediates ovine endometrial prostaglandin F2alpha synthesis: role of G(i) proteins and mitogen-activated protein kinases. Biol. Reprod. **65:** 1150–1155.
57. GARCIA VILLAR, R., P.L. TOUTAIN, D. SCHAMS, *et al.* 1983. Are regular activity episodes of the genital tract controlled by pulsatile releases of oxytocin? Biol. Reprod. **29:** 1183–1188.
58. OKUDA, K., A. MIYAMOTO, H. SAUERWEIN, *et al.* 1992. Evidence for oxytocin receptors in cultured bovine luteal cells. Biol. Reprod. **46:** 1001–1006.
59. KHAN-DAWOOD, F.S., J. YANG & M.Y. DAWOOD. 1995. Potential role of oxytocin in cell to cell communication in the corpus luteum. Adv. Exp. Med. Biol. **395:** 507–516.
60. KNAUS, H. 1950. Die Physiologie der Zeugung des Menschen. Third edition. Wilhelm Maudlach Verlag. Wien. 34.
61. DARCONZA, G., S. PARADISO, R. TRENTADUE, *et al.* 1990. Electrical activity of the human uterus *in vivo* in the phases of the menstrual cycle. Clin. Exp. Obstet. Gynecol. **17:** 171–180.
62. VERDENIK, I., M. PAJNTAR & B. LESKOSEK. 2001. Uterine electrical activity as predictor of preterm birth in women with preterm contractions. Eur. J. Obstet. Gynecol. Reprod. Biol. **95:** 149–153.
63. KUNZ, G., M. HERBERZT, M. NOE, *et al.* 1998. Uterine peristalsis during the follicular phase of the menstrual cycle: effects of oestrogen, antioestrogen and oxytocin. Hum. Reprod. Update **4:** 647–654.
64. BAIRD, D.T. & I.S. FRASER. 1975. Concentration of oestrone and oestradiol in follicular fluid and ovarian venous blood of women. Clin. Endocrinol.. **4:** 259–264.
65. KHAN-DAWOOD, F.S., L.T. GOLDSMITH, G. WEISS, *et al.* 1989. Human corpus luteum secretion of relaxin, oxytocin, and progesterone. J. Clin. Endocrinol. Metab. **68:** 627–631.
66. GINTHER, O.J. 1974. Internal regulation of physiological processes through local venoarterial pathways. A review. J. Anim. Sci. **39:** 550–564.
67. CZERKWINSKI, J. 1985. Selected problems of morphology of the venous system of the female internal genital organs. II. Morphology of the veins of the mesosalpinx. The venous system in cases of uterine myoma. Atypical anastomoses between ovarian and uterine venous trunks. Ginekol. Pol. **56:** 293–299.
68. EINER JENSEN, N. 1990. Functional and therapeutic aspects of counter current transfer in the female adnexa. Med. Hypotheses **33:** 125–127.
69. KRZYMOWSKI, T., J. KOTWICA & S. STEFANCZYK KRZYMOWSKA. 1990. Uterine and ovarian countercurrent pathways in the control of ovarian function in the pig. J. Reprod. Fertil. Suppl. **40:** 179–191.
70. STEFANCZYK KRZYMOWSKA, S., J. CHLOPEK, W. GRZEGORZEWSKI, *et al.* 2005. Local transfer of prostaglandin E2 into the ovary and its retrograde transfer into the uterus in early pregnant sows. Exp. Physiol. **90:** 807–814.

Exhibit 73

Retrograde migration of glove powder in the human female genital tract

A.C.E.Sjösten[1], H.Ellis[2] and G.A.B.Edelstam[1]

[1]Karolinska Institutet, Department of Obstetrics & Gynaecology at Stockholm Söder Hospital, s-118 83 Stockholm, Sweden and[2]Department of Anatomy, Guy's, King's and St Thomas' School of Biomedical Sciences, London Bridge, London SEI 9RT, UK

[3]To whom corespondence should be addressed. E-mail: anette.sjosten@sos.sll.se

BACKGROUND: This study in humans was undertaken to evaluate earlier results from animal research showing a retrograde migration of glove powder from the vagina into the intra-abdominal cavity. METHODS: One study group was gynaecologically examined with powdered gloves the day before an abdominal hysterectomy and another group 4 days pre-operatively. There were two control groups similarly examined with powder-free gloves. Cell smears were taken from the peritoneal fluid and during the operation further smears were taken from the Fallopian tubes, uterine cavity and cervical canal. RESULTS: Statistically significant differences were found for large starch particles at all locations between the study and control groups examined 1 day pre-operatively. Considering small starch particles, there were significant differences in cervix ($P < 0.001$), uterus ($P < 0.01$) and the Fallopian tubes ($P < 0.01$). The combined results also show significant differences between both large and small starch particles in cervix, uterus and the Fallopian tubes. There were also differences between the study and control groups examined 4 days pre-operatively, but these were not statistically significant except for small and large starch particles in uterus ($P < 0.01$, $P < 0.05$) and cervix ($P < 0.05$, $P < 0.05$). CONCLUSIONS: This study has pointed out a retrograde migration of starch also in humans after a gynaecological examination with powdered gloves. Consequently, powder or any other potentially harmful substance that can migrate from the vagina should be avoided.

Key words: female/gloves/retrograde migration/starch particles/vaginal examination

Introduction

Earlier case reports suggest that intra-abdominal granulomas or adhesions due to starch particles were caused by starch powder used on gloves during vaginal examination. An initial indication of retrograde flow through the Fallopian tubes was the finding of intraperitoneal starch granulomas (Paine and Smith, 1957). Later the first case of starch peritonitis in a patient without previous surgery was reported (Saxen *et al*., 1963). A recent investigation detected talcum particles on the ovaries in women who had used perineal talc applications (Heller *et al*., 1996). In contrast, tubal ligation prevents the access of mediators that reach the peritoneal cavity through the Fallopian tubes (Ylikorkala, 2001).

Powder-free gloves have been available for 20 years, but starch-powdered gloves are still available and in use (Sjösten *et al*., 1999).

It is well documented that starch-powdered gloves are not appropriate for abdominal surgery (Ellis, 1990; Holmdahl *et al*., 1994), and intraperitoneally, starch particles can initiate inflammatory reaction and the formation of adhesions (Edelstam *et al*., 1992; diZerega, 1994), although the mechanism by which starch increases the propensity of tissues to form adhesions is not known. Reduced peritoneal fibrinolysis and activation of leukocytes by particulate starch granules have been suggested as possible mechanisms. Activated leukocytes, particularly macrophages, produce supernormal amounts of oxygen-free radicals, prostaglandin E_2, thromboxane B_2 and various cytokines (Osman and Jensen, 1999). Starch particles also increase the eicosanoid production which may contribute to the inflammatory or immune reactions and development of adhesions (Chegini and Rong, 1999). If already injured mesotelial surface of the peritoneum is exposed to starch, more dense adhesions are created compared to the effect of peritoneal trauma or starch separately. Application of glove powder on minimally or severely traumatized peritoneum facilitates tumour cell adhesion and growth alone (van den Tol *et al*., 2001). Histological re-evaluation after tubal reconstructive surgery due to peritubal or peri-ovarian adhesions has shown residual starch from powdered gloves (Yaffe *et al*., 1980).

A causal connection has been shown between operative tissue damage, intra-abdominal ischaemia, infections, reactions to foreign materials such as sutures, particles of gauze, glove dusting powder and post-operative adhesions

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(Myllärniemi, 1967; Holmdahl *et al*., 1996). One of the proven causes of post-operative intestinal adhesions is microscopic foreign bodies which are present in up to 93% of adhesions (Duron *et al*., 1997). After open abdominal or pelvic surgery, a third of the patients are readmitted at least twice during the subsequent 10 years for a disorder directly or possibly related to adhesions (Ellis *et al*., 1999).

Our previous investigation in a rabbit model indicated a retrograde migration of glove powder from the vagina into the intra-abdominal cavity (Edelstam *et al*., 1997). The amount that reaches the peritoneum is sufficient to significantly increase formation of post-operative adhesions after a standardized trauma (Sjösten et el., 2000).

Therefore, this subsequent study in humans was done to investigate whether starch particles from powdered gloves also in humans might gain access to the abdominal cavity through the vagina after a gynaecological examination with powdered gloves.

Materials and methods

Patients

The participants in the study were divided into four different groups. Informed consent was obtained from all participants. All had a routine gynaecological examination before an elective laparotomy for total or subtotal hysterectomy due to fibroids or menometrorrhagia. Group I: examined 1 day pre-operatively with (i) powdered gloves (Gammex® Ansell GmbH, Germany; $n = 17$, mean age 51 years) or (ii) powder-free gloves (Biogel® Regent Medical, SLL) ($n = 15$, mean age 51 years). Group II: examined 4 days pre-operatively with (i) powdered gloves ($n = 12$, mean age 53 years) or (ii) powder-free gloves ($n = 14$, mean age 52 years). Patients with cancer of the uterus were excluded as well as women with ongoing menstrual bleeding. The pre-menopausal women were examined regardless of the follicular or luteal phase of the menstrual cycle. A third of all women in the study were post-menopausal. Any medication that might have influenced the tubal patency had not been taken except in the case of three patients who had an asthmatic disease and needed to take terbutaline occasionally. The medication was not taken during the investigations. There were no other significant differences for patient characteristics. Sexual activity, cyclic changes or hormonal effect where not considered in this study.

Surgical procedure

An abdominal subtotal or total hysterectomy was undertaken with the operating team and the nurse who set up the instrument tray wearing powder-free gloves. Immediately the abdominal cavity was opened, peritoneal fluid was collected and cell smears were then taken from the peritoneal fluid. From the fimbriae of the Fallopian tubes, additional cell smears were taken per-operatively and when the uterus had been removed, i.e. post-operatively from the uterine cavity and the cervical canal. For making the smears sterile, forceps or peans were used. Smears from the fimbriae of the Fallopian tubes were omitted if they were not removed during the hysterectomy.

Cell smears

The cell smears were quantitatively standardized on ~1 cm² of one-half of a glass slide with the other blank side serving as control for contamination with air-borne starch particles. All the slides were stained with May–Grünewald Giemsa by a biochemical assistant wearing powder-free gloves in a laboratory where only powder-free

Table I. Small and large starch particles on day 1 after examination with powdered (Ia) and powder-free (Ib) gloves respectively

		No. of patients	Total no. of particles	Median	Range	Mean	P
Cervix							
Small	Ia	17	70	1	14	4.1	< 0.001
	Ib	15	0	0	0	0	
Large	Ia	17	46	0	24	2.7	< 0.01
	Ib	15	1	0	1	0.01	
Uterus							
Small	Ia	17	104	2	48	6.1	< 0.01
	Ib	15	0	0	0	0	
Large	Ia	17	22	0	10	1.3	< 0.01
	Ib	15	1	0	1	0	
Fallopian tubes							
Small	Ia	12	34	1.5	16	2.8	< 0.01
	Ib	13	0	0	0	0	
Large	Ia	12	18	0	10	1.5	< 0.05
	Ib	13	0	0	0	0	
Peritoneal fluid							
Small	Ia	13	13	1	4	1.0	NS
	Ib	13	3	0	3	0.2	
Large	Ia	13	12	0	6	0.9	< 0.05
	Ib	13	0	0	0	0	

NS = not significant.

gloves were used. The slides were coded and analysed by two independent investigators with a Zeiss 4/76 microscope using polarized light at magnification ×250. The starch particles were counted in a standardized procedure for all slides. The numbers on the blank side (i.e. contamination) were subtracted from that in the smears so that the number of starch particles on each slide represent the net number without contaminating particles. Since there are differences in the size of starch particles they where divided into two sizes: (i) smaller than a leukocyte and (ii) larger than a leukocyte. Leukocytes for comparison in size were always present in the smears. The study was approved by the local ethics committee.

Statistics

Non-parametric Mann–Whitney U-tests and Fisher's exact test were used and values are given as SEM for the group. Differences were considered significant at the $P < 0.001$, $P < 0.01$ and $P < 0.05$ levels. All statistical tests were computerized and carried out with statistics programs (Statistica™; Statsoft, USA).

Results

Group I: examined 1 day pre-operatively with (i) powdered gloves (n = 17) and (ii) powder-free gloves (n = 15)

Starch particles were found in the cell smears with more particles found on the slides from the patients examined with powdered gloves. The differences were significant at all locations in the genital tract for small particles (cervix $P < 0.001$, uterus and Fallopian tubes $P < 0.01$) and large particles (cervix and uterus $P < 0.01$ and Fallopian tubes $P < 0.05$) but only for large particles in the peritoneal fluid ($P < 0.05$). However, in two patients examined with powdered gloves, no particles were found. On the contrary, in three patients examined with powder-free gloves, a few particles were found (Table I and Figure 1).
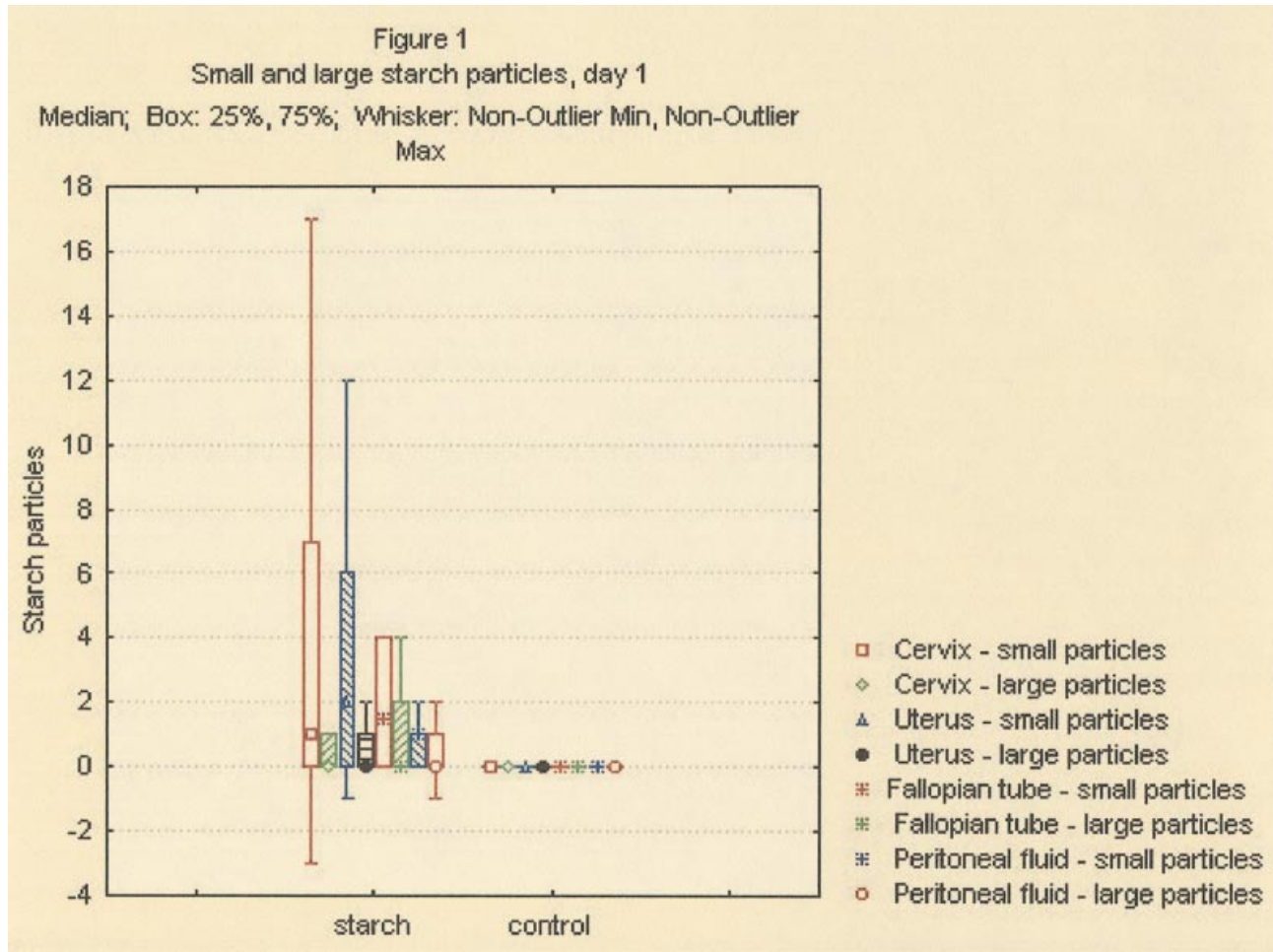
Figure 1. Median and range value for the retrograde transportation of small and large starch particles respectively, in different locations 1 day after a gynaecological examination with or without powdered gloves. The negative range value in the starch group for cervix, uterus and peritoneal fluid are due to contamination with airborne starch particles.

Group II: examined 4 days pre-operatively with (i) powdered gloves (n = 12) and (ii) powder-free gloves (n = 14)

There were significantly more small starch particles as well as large particles (cervix and uterus $P < 0.05$) after examination with powdered gloves. The differences were the same for small particles but less significant for large particles (uterus $P < 0.05$). The differences were non-significant in the Fallopian tubes and the peritoneal fluid (Table II and Figure 2.

Discussion

Medical gloves for use in surgery were introduced in 1896. Since then, several additives have been tried to facilitate manufacturing and to reduce the hazards associated with glove use (Ellis, 1990). Rubber and glove lubricants are the two main components in modern gloves. Starch powder as a glove lubricant can lead to complications such as granulomatous peritonitis (Giercksky *et al.*, 1994), adhesion formation (van den Tol *et al.*, 2001) and potentiation of infection (Renz and Gemsa, 1997), with subsequent intestinal obstruction, infertility and chronic pelvic pain.

Table II. Numbers of small and large starch particles after examination with powdered (IIa) and powder-free (IIb) gloves respectively, day 4

			No. of patients	Total no. of particles	Median	Range	Mean	*P*
Cervix								
Small	IIa	12	26	1	2	2.1	< 0.05
	IIb	14	0	0	0	0	
Large	IIa	12	9	0	3	0.8	< 0.05
	IIb	14	0	0	0	0	
Uterus								
Small	IIa	12	21	3	20	1.8	< 0.01
	IIb	14	2	0	0	0.1	
Large	IIa	12	7	0	3	0.6	< 0.05
	IIb	14	0	0	0	0	
Fallopian tubes								
Small	IIa	11	16	1	5	1.4	NS
	IIb	14	4	0	1	0.2	
Large	IIa	11	2	0	1	0.2	NS
	IIb	14	0	0	0	0	
Peritoneal fluid								
Small	IIa	9	14	1	5	1.6	NS
	IIb	11	3	0	1	0.3	
Large	IIa	9	2	0	1	0.2	NS
	IIb	11	0	0	0	0	

NS = not significant.

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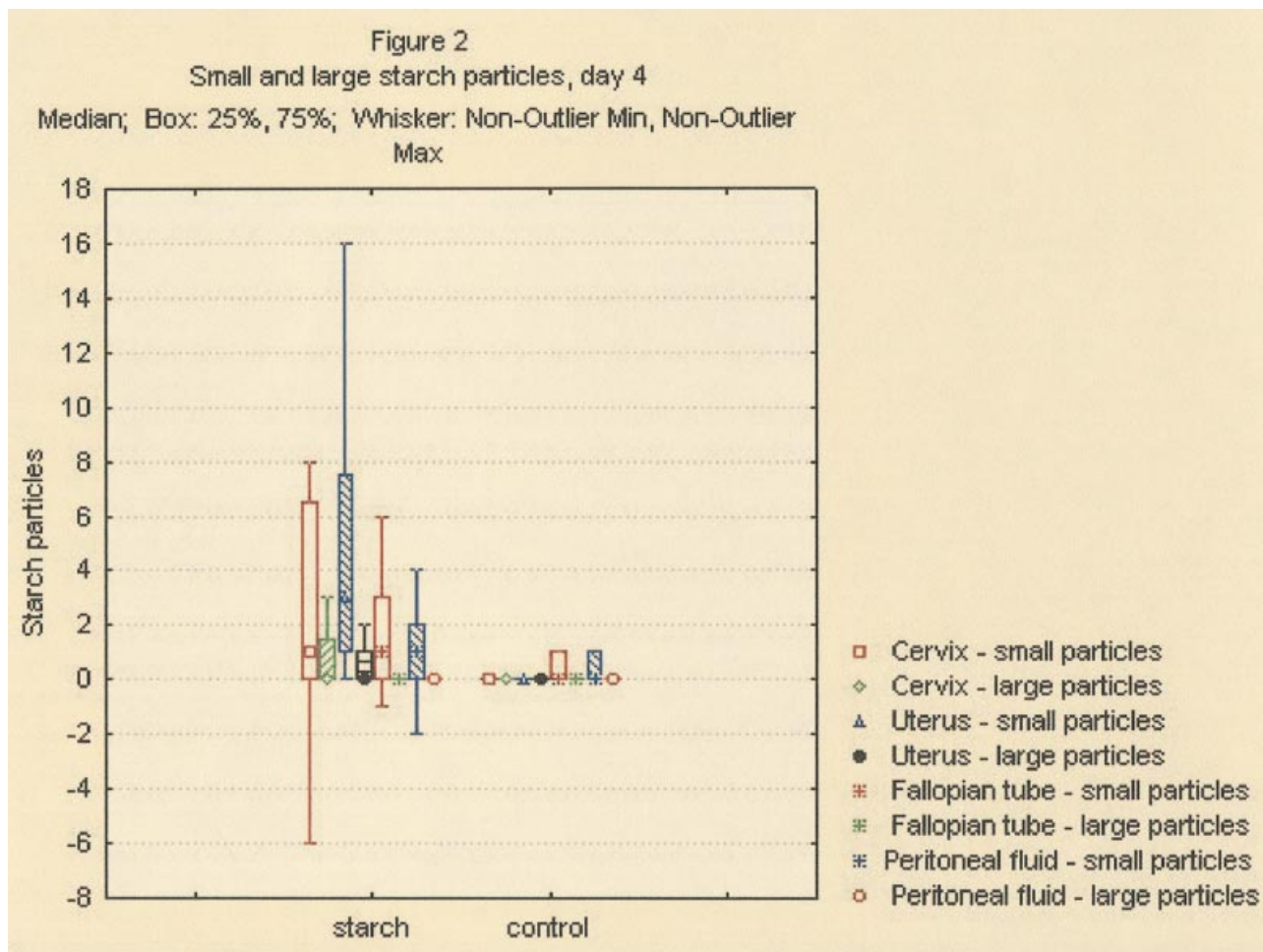**A.C.E.Sjösten, H.Ellis** and **G.A.B.Edelstam**

Figure 2. Median and range value for the retrograde transportation of small and large starch particles respectively, in different locations 4 days after a gynaecological examination with or without powdered gloves. The negative range value in the starch group for cervix, Fallopian tube and peritoneal fluid are due to contamination with airborne starch particles.

The possibility of retrograde migration of starch particles in the female genital tract into the intraperitoneal cavity has been suspected for several decades (Saxen *et al.*, 1963). The present study in humans has attempted to investigate whether previous results from animal research—that starch particles can migrate from the vagina into the abdominal cavity (Edelstam *et al.*, 1997)—reflects the case in humans. This study indicates such a retrograde migration of starch particles after gynaecological examination with powdered gloves. There were statistically significant differences between study and control groups in cervix, uterus and Fallopian tubes on the first day after vaginal examination with powdered gloves compared to powder-free examination. The low number of starch particles in the cell smear of the peritoneal fluid may reflect differences in the total amount of fluid and that it might have been better to collect all the fluid and after centrifugation prepare cell smears. However, with the present approach a significant difference between pre-operative examination with powdered and powder-free was demonstrated. The lower number of particles on the fourth day might indicate that absorption of starch particles had started, or that the particles had adhered to the peritoneum. In previous animal studies, most particles were found on the third day after

deposition in the vagina (Edelstam *et al.*, 1997). The numbers found in the controls indicate that the presence of starch particles in the peritoneal cavity is in accordance with reported persistance for up to 18 months (Ellis, 1971). Our present patients have been examined in that time before the referral for hysterectomy.

A considerable number of gynaecologists wears starch-powdered gloves (Sjösten *et al.*, 1999), despite evidence of starch-induced complications. The starch particles can migrate not only from the vagina into the cervical canal and the uterine cavity but also through the Fallopian tubes into the peritoneal fluid. Women exposed to intra-abdominal surgical trauma 1–4 days after a gynaecological examination with powdered gloves may be at increased risk of intra-abdominal adhesions. But even without a surgical procedure there is a risk of intra-abdominal or peri-tubal adhesions due to the examination with powdered gloves (Osser *et al.*, 1989). Ongoing subclinical PID can cause infective tissue damage. An extensive study by Myllärniemi (1967) showed that talc, starch powder and lint in the abdominal cavity tended to accumulate in the traumatized areas of the peritoneum so that the foreign material contaminating the peritoneal tissues could act together with other

traumatizing conditions, possibly preventing the resorption of fibrinous adhesions. This corresponds to our previous finding in the rabbit model that starch particles deposited in the vagina can migrate in a retrograde direction from the vagina into the abdominal cavity and, combined with an intra-abdominal trauma, generate dense adhesions (Sjösten *et al.*, 2000). Since there are indications towards retrograde migration of powder, it must not be used regardless of cyclic variations or sexual activity.

In conclusion, our results show that starch particles can migrate from the vagina into the cervical canal, the uterine cavity and through the Fallopian tubes up to 4 days after a gynaecological examination with powdered gloves. Glove powder contributes to adverse intra-abdominal reactions, which include adhesion formation and adhesion-related complications such as chronic pelvic pain and bowel obstruction. Tubal and pelvic adhesions are a major cause of female infertility. Since evidence suggests that a retrograde migration could be a general mechanism, our recommendation is that we should be critical of harmful substances, e.g. glove powder, that could migrate from the vagina to abdominal cavity.

Acknowledgements

References

Chegini N and Rong H (1999) Postoperative exposure to glove powders modulates production of peritoneal eicosanoids during peritoneal wound healing. Eur J Surg 165,698–704.

di Zerega GS (1994) Contemporary adhesion prevention. Fertil Steril 61,219–235.

Duron JJ, Ellian N and Olivier O (1997) Post-operative peritoneal adhesions and foreign bodies. Eur J Surg 579(Suppl),15–16.

Edelstam GAB, Lundkvist E., Laurent TC et al (1992) The concentration and turnover of intraperitoneal hyaluronan during inflammation. Inflammation 16,459–469.

Edelstam GAB, Sjösten ACE and Ellis H (1997) Retrograde migration of starch in the genital tract of rabbits. Inflammation 21,489–499.

Ellis H (1971) The cause and prevention of postoperative intraperitoneal adhesions. Surg Gynecol Obstet 133, 497–511.

Ellis H (1990) The hazards of surgical glove dusting powder. Surg Gynecol Obstet 171,521–527.

Ellis H, Moran JB, Thompson NJ et al (1999) Adhesion-related hospital readmissions after abdominal and pelvic surgery: a retrospective cohort study. Lancet 9163,1476–1480.

Giercksky KE, Qvist H, Nesland TM et al (1994) Multiple glove powder granulomas masquerading as peritoneal carcinomatosis. J Am Coll Surg 179,299–304.

Heller DS, Westhoff C, Katz N et al (1996) The relationship between perineal cosmetic talc usage and ovarian talc particle burden. Am J Obstet Gynecol 174,1507–1510.

Holmdahl L, Al-Jabreen M and Risberg B (1994) Experimental models for qantitative studies on adhesion formation in rats and rabbits. Eur Surg Res 26,248–256.

Holmdahl L, Al-Jabreen M and Risberg B (1994) The impact of starch-powdered gloves on the formation of adhesions in rats. Eur J Surg 160,257–261.

Myllärniemi H (1967) Foreign material in adhesion formation after abdominal surgery. Acta Chir Scand 377,1–48.

Osman MO and Jensen SL (1999) Surgical gloves: current problems. World J Surg 23,630–637.

Osser S, Persson K and Liedholm P (1989) Tubal infertility and silent chlamydial salpingitis. Hum Reprod 4,280–284.

Paine CG and Smith P (1957) Starch granulomata. J Clin Pathol 10,51–55.

Renz H and Gemsa D (1997) Effects of powder on infection risks and associated mechanisms. Eur J Surg 579(Suppl),35–38.

Saxen L, Kissinen A. and Saxen E (1963) Peritoneal foreign-body reaction caused by condom emulsion. Lancet 2,1295–1296.

Sjösten ACE, Blomgren H and Edelstam GAB (1999) Precautions taken to prevent adhesions—a questionnaire study among Swedish obstetricians and gynaecologists. Eur J Surg 165,736–741.

Sjösten ACE, Ellis H and Edelstam GAB (2000) Post-operative consequences of glove powder used pre-operatively in the vagina in the rabbit model. Hum Reprod 15,1573–1577.

van den Tol M.P., Haverlag R, Jeekel J et al (2001) Glove powder promotes adhesion formation and facilitates tumour cell adhesion and growth. Br J Surg 88,1258–1263.

Yaffe H, Beyth Y and Levij IS (1980) Foreign body granulomas in peritubal and periovarian adhesions: a possible cause for unsuccessful reconstructive surgery in infertility. Fertil Steril 33,277–279.

Ylikorkala O (2001) Tubal ligation reduces the risk of ovarian cancer. Acta Obstet Gynecol Scand 80,875–877.